Assessment of Exposure to Sensitizing Rosin-derived Compounds from Electronics Soldering

Final Report

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Introduction

The objective of this research project was to assess the exposure to rosin-derived compounds, which include volatile and semivolatile organic compounds, produced during electronics soldering operations using rosin-based fluxes and rosin core solders. Soldering has been previously determined to be a risk factor for development of occupational asthma, and little work had been done to characterize compounds produced by heating rosin flux to soldering temperatures (350-400°C). A reaction system was constructed which allowed for a continuous flow of air passing over a solder iron tip and which carried emissions through a glass fiber filter (or other collection medium such as a sorbent tube) for subsequent trapping and analyses of emission products. Particulate matter was collected on a filter, while the more volatile organic compounds were collected on the sorbent tube media for analysis by gas chromatography/mass spectrometry (GC/MS). Comparisons were made between samples collected using the reaction system and actual breathing zone samples collected during soldering operations.

The particulate produced by heating rosin flux was characterized by several of the collaborating researchers, prior to ONR funding of this work. In that work, it was shown that the particulate consists principally of diterpene resin acid compounds such as dehydroabietic acid. Also observed were oxidized resin acids, which were present in lower proportions. The principal oxidized resin acid observed in laboratory and field samples was 7-oxodehydroabietic acid, a compound known to be a sensitizer.

Subsequent work funded by the ONR grant explored:

1. A protein conjugation or haptenation mechanism for 7-oxodehydroabietic acid, an oxidized resin acid compound shown to be present in aerosol produced by heating rosin flux. A likely explanation for this compound's sensitizing ability may be its ability to bind to lysine via

formation of a Schiff base or imine linkage as demonstrated by *in vitro* experiments.

2. Low molecular weight aldehydes were identified using gas chromatography/mass spectrometery (GC/MS). Previous GC studies using a flame ionization detector had shown only three aldehydes produced during heating of rosin flux, but the sensitivity and structural information provided by MS gave evidence of an additional eight aldehydes in rosin emissions. At least two of these aldhydes (formaldehyde and acetaldehyde) are suspected carcinogens and all those detected are likely to cause pulmonary irritation. In addition to the aldehydes, other chemical compound groups such as terpenes, ketones, and carboxylic acids were also found.

3. Resin acid metabolism was studied *in vitro* to identify mammalian metabolites produced from dehydroabietic acid, a resin acid compound commonly observed in aerosol samples produced from heated rosin flux. Two metabolites were observed in the *in vitro* rat liver homogenate system used in the studies: abieta-8,11,13,15-tetraen-18-oic acid, and 15-hydroxydehydroabietic acid.

4. A rapid method for analyzing headspace sampling of aldehydes was investigated using a relatively new analytical technique, solvating gas chromatography (SGC). This technique was used to separate the aldehyde compounds studied with enhanced selectivity and resolving power compared to conventional open tubular gas chromatography.

To summarize, this project provided information regarding the specific compounds generated during rosin heating in electronic soldering applications. A possible biological mechanism was shown for sensitization to a resin acid present in aerosol produced during heating rosin flux. The work described may prove useful in producing an exposure standard for contaminant sampling during electronics soldering operations.

2 Results and Discussion

2.1 Oxidized Resin Acids in Aerosol Derived from Rosin Core Solder[1]

2.1.1 Abstract

Exposure to rosin during a variety of uses has been associated with dermal and pulmonary sensitization. Oxidized resin acids are present in many rosin products, and have been regarded as the main sensitizing rosin compounds in cases of dermal sensitization. This research describes oxidized resin acids identified in aerosol produced during soldering with rosin core solder. Oxidized resin acids found were 7-oxodehydroabietic acid, 15-hydroxydehydroabietic acid, and 7-hydroxydehydroabietic acid. The presence of oxidized compounds known to be dermal sensitizers in aerosol from rosin flux soldering supports the hypothesis that resin acid compounds are pulmonary sensitizers as previously proposed. Changes in the composition of resin acid aerosol derived from heated rosin core solder (compared to the parent material) are described.

2.1.2 Introduction

Rosin flux is widely used in electronics soldering, both as a solder constituent (rosin flux-core solder) and as a liquid flux (usually rosin dissolved in isopropanol). The handling and use of rosin have been associated with both allergic contact dermatitis and occupational asthma, as described by Sadhra *et al.* in a recent review.[1] The dermal sensitizing capability of resin acids has been shown to result from contact with oxidized species often present in the solid rosin to which sensitized subjects have been exposed.[2-7] The dermal sensitizing capacity of hydrogenated resin acids (which are less readily susceptible to air oxidation) has been shown to be reduced.[8] Resin

[1]Authored by P.A. Smith, D.R. Gardner, D.B. Drown, W.W. Jederberg, and K. R. Still

acids, including apparently oxidized resin acids, have been described in respirable aerosols collected during soldering with rosin flux.[9] Pulmonary sensitization (presumably to resin acid aerosol) has been described in a rosin core solder manufacturing facility where the rosin was heated to a point insufficient to produce thermal decomposition products (gas and vapor contaminants such as formaldehyde).[10] Also, workers exposed to unheated rosin dust[11] and a mist containing rosin[12] have developed pulmonary sensitization to rosin. A hypothesis that pulmonary sensitization seen in some electronics solderers using rosin flux is due to exposure to respirable resin acids logically follows.

Aerosol filtration and extraction followed by gas chromatographic/mass spectrometric (GC/MS) analytical methodologies were used to confirm the presence and identities of oxidized resin acids present in aerosol derived from electronics soldering with rosin core solder. Changes in resin acid composition, when comparing the resin acids seen in rosin core solder derived aerosol to the unheated flux material, were also investigated with gas chromatography/mass spectrometry (GC/MS) methodology.

2.1.3 Materials and Methods

2.1.3.1 Personal Samples

Personal air samples were collected from the breathing zone of individual students performing electronics soldering in an electronics training facility. Eighteen samples were collected at this facility over a period of 10 months. Several samples taken during production soldering of printed circuit boards with rosin core solder were used for comparison. Different commercial solder sources were used for the two types of samples and no liquid flux was used during either type of soldering. The samples, although collected over the full exposure period when soldering was

performed, were of short duration (1 hour or less with training samples, 2 hours or less for production samples) with intermittent to continuous soldering taking place. There was no local exhaust ventilation used during soldering activities at either operation.

OSHA Method 58[13] (coal tar pitch volatiles, coke oven emissions, and polynuclear aromatic hydrocarbons) sampling and analytical procedures were followed, with several modifications to extraction volumes and other aspects of the method discussed previously.[9] Method 58 has been shown to be useful in collecting resin acid aerosol, as the resin acids remain in the solid phase as an aerosol following initial volatilization. The resin acid compounds thus trapped are easily extracted from the collection filter with a suitable solvent.[9,14] The most significant deviations from Method 58 procedures were that methylene chloride (analytical reagent grade, Mallinckrodt, Paris, KY) was used instead of benzene to extract the samples, and glass fiber filters (GFFs, SKC, Inc., Eighty Four, PA) used to collect samples were pre-cleaned by overnight soxhlet extraction with methylene chloride. Immediately upon completion of sample collection, each filter was transferred to a clean 25-mL glass vial having a polytetrafluoroethylene (PTFE) lined lid and aluminum foil wrapping. Field sample vials with filters were then taken directly to the laboratory, and each vial was flooded with N_2, sealed and kept at room temperature overnight while awaiting analysis.

Personal sampling pumps (Gilian, Model Gilair5, St. Louis, MO) were used to collect personal samples, and were calibrated before and after sampling to ensure that post-sampling flow rates were maintained within ±5% of the presampling rates. Sampling flow rates ranged from 1.8 to 2.0 liters per minute.

A sample of the rosin core solder used in the electronics training facility was collected for extraction of the unheated flux, which provided a comparison to the corresponding air samples.

2.1.3.2 Flux Extraction and Laboratory Heating

From the same eutectic lead/tin (Pb/Sn) rosin core solder source used in the personal samples, bulk samples were cut, and replicates were produced using the following methods: (1) Three lengths of Pb/Sn rosin core solder (6.0 cm each) were split with a razor blade and extracted with methylene chloride for 15 minutes. (2) Three samples were prepared using the above extraction procedure, followed by solder-free heating of the methylene chloride extracts on a soldering iron tip maintained between 340 and 360 °C. The heating was accompanied by simultaneous collection of the aerosol produced for each replicate. (3) Three samples were made with direct heating of 6.0-cm lengths of intact Pb/Sn rosin core solder on a soldering iron tip maintained between 340 and 360 °C. The heating was accompanied by simultaneous collection of the aerosol produced for each replicate. (4) Three samples were produced as per (3) above, but were then subjected to prolonged sampling by running the sampling pump at 2 liters per minute with the sampling cassette and loaded filter in place for 150 ± 5 minutes following collection of each sample. This essentially duplicated the sampling flow rate used in personal samples, and exceeded the duration of all personal samples collected.

The same sampling pumps used to collect personal samples were also used to collect laboratory-generated aerosol. The pumps were operated at 4 liters per minute during aerosol loading. Flux extracts were slowly injected onto the heated soldering iron tip with a 10-μL syringe, (Hamilton, Reno, NV) and the aerosol generated was directed towards the filter by a cowling made of extra 3-piece sampling cassette "middle" sections, which extended the zone of undisturbed air in front of the filter by about 2 inches. The tip of the soldering iron was kept within ½ inch of the GFF face inside the cowling area, while flux extract or rosin core solder was heated. For rosin core solder heating, the length of solder to be heated was slowly applied to the heated soldering iron tip using

tweezers. The 3-piece styrene acrylonitrile cassettes used were standard 37-mm units (Gelman Sciences Inc., Ann Arbor, MI) used with the same GFFs as for personal samples. All samples were extracted immediately following sampling. Prolonged sampling filters were extracted immediately following the prolonged sampling period. All filter extracts of laboratory generated samples were kept in the dark below 0 °C in a standard freezer while awaiting derivatization and analysis, which occurred on the day following sample creation.

2.1.3.3 GC/MS Methods

A Finnigan MAT GCQ GC/MS instrument equipped with a 30-m DB-5 MS capillary column (J&W Scientific, Folsom, CA) and split/splitless injection was used. The column inside diameter was 0.25 mm, and the stationary phase thickness was 0.25 μm. The carrier gas was helium, with a constant velocity flow rate of 40 cm/sec. For all samples, column temperature was maintained at 140 °C for 1.0 minute and then increased 40 °C/minute to 200 °C, and then 5 °C/minute to 300 °C.

Personal samples collected in the field were analyzed using splitless injection mode to increase analytical sensitivity for filters which were lightly loaded. Laboratory-generated samples were more heavily loaded and were analyzed using the split mode, with a split ratio of 20:1. The injector temperature was kept at 250 °C. The GC/MS transfer line was maintained at 275 °C. Electron ionization (70 eV) was used for all samples, with an ion source temperature of 150 °C. Scan rate was 0.5 seconds/scan and mass spectra were collected over the mass-to-charge range 50-650 *m/z*.

2.1.3.4 Extraction and Solvent Transfer

Samples were prepared for derivatization and GC/MS analysis following procedures previously reported.[9] Following extraction with methylene chloride and filtration to remove any glass fibers, a 0.5-mL aliquot of extract was taken for GC/MS analysis. This sample was evaporated to dryness using a stream of N_2 at 70 °C. Dimethylformamide (DMF, 0.5 mL, Mallinckrodt, analytical reagent grade), containing heptadecanoic acid (Sigma, St. Louis, MO) as an internal standard, was then added.

2.1.3.5 Sample Derivatization/Analysis

Carboxylic acid groups present in the samples were esterified by addition of a methyl group to allow chromatographic separation of resin acid compounds. Methyl esterification also allowed comparison of observed mass spectra with pertinent resin acid methyl ester reference spectra found in the literature.[7,15-19] Conversion to methyl esters was accomplished by adding excess potassium carbonate (20-30 mg, reagent grade, MCB, Norwood, OH), and 50.0 μL of methyl iodide (99% purity, Fisher, Pittsburgh, Pa.), followed by gentle shaking for about 30 minutes. This method for conversion to methyl esters was shown to go essentially to completion by high performance liquid chromatography/mass spectrometry (HPLC/MS). No free resin acid was detected in a 100-μg sample of dehydroabietic acid subjected to the derivatization procedure, and an experiment with a mixture of derivatized and nonderivatized dehydroabietic acid showed that free resin acid levels of 1% would be detectable by the HPLC /MS method.

Following analysis, the resulting GC/MS chromatogram peaks were integrated, and the dehydroabietic acid methyl ester and abietic acid methyl ester ratios were calculated. The resulting ratios of different replicate groups were subjected to statistical analysis using two-tailed *t*-tests (equal

and unknown variance).

Dehydroabietic acid (>99% purity), abietic acid (95% purity), and 7-oxodehydroabietic acid (95% purity) were purchased for reference standards (Helix Biotech, Richmond, BC, Canada). An authentic sample of 15-hydroxydehydroabietic acid methyl ester was synthesized using the methodology of Shao *et al.*,[7] with the structure confirmed using infrared spectroscopy, ^{1}H nuclear magnetic resonance, and mass spectrometric methods.

Individual resin acid compounds were identified as the methyl esters by relative retention times (dehydroabietic acid = 1.00) and mass spectra comparison to either standard samples, or to those previously reported.[7, 15-19] The EI (70 eV) MS data obtained for the reference standards and other resin acid methyl esters observed are listed in Table 2.1.1 as *m/z* (relative intensity).

2.1.4 Results

2.1.4.1 GC/MS Analyses

The personal sample GC/MS chromatograms were examined to identify the oxidized resin acid compounds present using retention time and mass spectral characteristics. Resin acids (oxidized and nonoxidized) were detected in personal samples from both electronics training and production soldering. A representative sample chromatogram of unheated flux material (which had a relatively large proportion of abietic acid compared to other resin acids normally present in rosin core solder) is shown in Figure 2.1.1A. A chromatogram of a personal sample collected with rosin core solder of the same source is shown in Figure 2.1.1B.

The principal resin acid most often detected in the personal samples was dehydroabietic acid. Oxidized resin acids detected include: 7-hydroxydehydroabietic acid, 15-hydroxydehydroabietic acid, and 7-oxodehydroabietic acid. Chemical structures of pertinent resin acids are provided in

Figure 2.1.2. The major resin acid of the unheated flux, abietic acid, was not readily detected in most of the personal samples, while the levels of oxidized resin acids (relative to nonoxidized resin acids) seen in several personal samples were much greater than in the parent material. The most common oxidized resin acid seen in breathing zone samples was 7-oxodehydroabietic acid. This compound was observed in all breathing zone samples where resin acids were detected.

The oxidized compounds were scarcely detectable in the unheated parent material, and were only seen above background when examining extracted ion chromatograms of masses indicative of such compounds. Oxidized resin acids were detected in the unheated parent material, although their levels were much lower than in many personal samples, both in absolute and relative terms.

2.1.4.2 Flux Extraction and Laboratory Heating

Table 2.1.2 summarizes data for laboratory generated samples. The average ratio of dehydroabietic acid to abietic acid increased compared to the unheated parent material when rosin was heated in the presence of Pb/Sn solder ($p<0.005$, $t = 8.05$, 4 d.f.). Prolonged air filtration of samples prepared by heating flux in the presence of Pb/Sn solder provided no significant ratio difference ($p>0.1$, $t = 1.20$, 4 d.f.) when compared to the same type of sample subjected to immediate extraction. The ratio in flux extract heated in the absence of Pb/Sn solder was also statistically indistinguishable ($p>0.1$, $t = 1.48$, 4 d.f.) from the unheated parent material.

2.1.5 Discussion

2.1.5.1 Oxidized Resin Acids

The observed oxidized compounds include several known to be dermal sensitizers such as 15-hydroxydehydroabietic acid[3] and 7-oxodehydroabietic acid.[4,6] The presence of these oxidized

species lends credibility to the hypothesis first proposed by Burge *et al.*[10] that resin acids present in flux-derived air contaminants are responsible for observed pulmonary sensitization in electronics solderers using rosin flux. Interestingly, all of the oxidized resin acids seen in our samples were based upon the dehydroabietic acid structure.

2.1.5.2 Preferential Recovery of Dehydroabietic Acid

In earlier work,[9] it was noted that dehydroabietic acid was the main resin acid observed in workplace samples collected during soldering with rosin core solder. Chromatographic peaks thought to be oxidized resin acids, and a near absence of abietic acid were also seen in those samples. The predominance of dehydroabietic acid, oxidized derivatives of dehydroabietic acid, and little abietic acid (relative to the parent material) were again seen in breathing zone samples collected during this investigation while using rosin core solder from another source.

The changes seen in dehydroabietic/abietic acid ratios in field samples were more drastic than for the laboratory-generated samples. The changes seen in field samples were probably not due to degradation or reactions on the filter, as the laboratory samples subjected to prolonged filtration showed no discernible difference compared to samples made with the same protocol yet without prolonged sampling. Also, it has been shown previously that abietic acid degrades on a filter kept in air contact with intermittent light exposure, taking 1 month to degrade by a factor of about one half.[9] The results seen in that study cannot explain the near absence of abietic acid on our personal sample filters, as our samples were analyzed 1 day after collection. Additionally, personal sample filters in the current study were maintained for most of the short time between collection and analysis in the dark, under nitrogen.

As reported previously by various investigators, the current exposure data appear to confirm

that dehydroabietic acid is more stable to thermal degradation than abietic acid, and/or that dehydroabietic acid may be formed from abietic acid by oxidation-dehydration reactions.[20-23] Such reactions could be hastened by the elevated temperatures of the soldering iron tip. Song *et al.* report that at elevated temperatures, and especially with a metal catalyst present, rosin is changed, with abietadienoic acids consumed, and dehydroabietic acid being the principal product.[21] However, the laboratory experiments reported here did not necessarily demonstrate that abietic acid was converted to dehydroabietic acid.

Another possibility that may explain the observed changes in abietic acid and dehydroabietic acid ratios is that abietic acid may be preferentially degraded by heating in the presence of Pb/Sn solder, resulting in a significant increase in the dehydroabietic acid recovered relative to abietic acid. For the purposes of exposure assessment, however, the data presented here and previously [9] clearly show that dehydroabietic acid, pimaric acid, isopimaric acid, and sandaracopimaric acids are probably the significant nonoxidized resin acid contaminants in aerosol derived from rosin core solder.

The persistence of dehydroabietic acid has been reported in the literature, and several references exist which parallel our observations concerning loss of abietic acid with no loss (and perhaps an increase) of dehydroabietic acid. Brownlee and Strachan[17] showed dehydroabietic acid to be persistent in waters tainted by pulp mill effluent (with no abietic acid seen in remote water samples), and recommended dehydroabietic acid as an indicator "of the areal influence of the effluent." Abietic acid was present in the source effluent stream, at levels greater than dehydroabietic acid. Zinkel[23] found dehydroabietic acid to persist in piles of loblolly pine chips, reporting that storage of loblolly chips in piles without chemical treatment resulted in partial losses of all resin acids, and formation of dehydroabietic acid. These previous studies do not provide an

answer as to why dehydroabietic acid is more persistent, but do indicate that under a variety of circumstances, dehydroabietic acid is probably more stable than abietic acid.

2.1.5.3 Stable Resin Acids as Potential Exposure Standards

In considering a relevant resin acid to use as an exposure standard, dehydroabietic acid and an oxidized compound such as 7-oxodehydroabietic acid, which were frequently observed in our personal samples derived from multiple rosin sources, may be suitable marker compounds to evaluate exposure to resin acid aerosol. Use of 7-oxodehydroabietic acid as such a standard would be warranted all the more if pulmonary exposure to the compound can be linked to pulmonary sensitization, as it has been linked to dermal sensitization.[3,4,6] However, additional work is necessary to determine the biological consequences of pulmonary exposure to this oxidized resin acid.

2.1.6 Conclusion

Oxidized dehydroabietic acid derivatives were detected in aerosol from soldering with rosin core solder. Two of these, 7-oxodehydroabietic acid and 15-hydroxydehydroabietic acid, have been shown by other researchers to be dermal sensitizers. A hypothesis that resin acid compounds are pulmonary sensitizers is strengthened by this finding.

The laboratory data cannot explain all of the changes observed in workplace sampling data, as the changes seen in dehydroabietic acid/abietic acid ratios were more severe with field samples. However, a statistically significant change in the dehydroabietic acid/abietic acid ratio was observed in the same direction when comparing laboratory-generated aerosol from rosin core solder to unheated extract from the same source. No additional changes were seen in this ratio from extended

air flow across the sampling filters, nor were changes seen when flux extract was heated in the absence of Pb/Sn solder. The observed changes could be explained by oxidation-dehydration changes in abietadienoic acids, leading to the more stable dehydroabietic acid. Another explanation for the observed data could be that the abietic acid is preferentially degraded to materials not detected by the sampling and analytical methods used, while dehydroabietic acid is simply more stable to thermal breakdown.

2.2 Conjugation of 7-Oxodehydroabietic Acid to Lysine, a Haptenation Mechanism for an Oxidized Resin Acid with Dermal Sensitizing Properties[1]

2.2.1 Abstract

This work explores protein conjugation of 7-oxodehydroabietic acid, a resin acid found in both aerosol from soldering with rosin flux and in rosin solids. In a murine model, conjugation (haptenation) of resin acids to proteins is required to generate antibodies against rosin. Hydroperoxy resin acids are dermal sensitizers, with haptenation thought to occur via radical mechanisms. Dermal sensitization to 7-oxodehydroabietic acid has been observed, although no radical haptenation mechanism has been proposed to explain the sensitizing properties of this compound. Conjugation of L-lysine to 7-oxodehydroabietic acid was predicted, with a Schiff base (or imine) linkage formed between C-7 of the resin acid and a free amino group of lysine. Fast atom bombardment mass spectrometry provided evidence of the conjugate; a small peak was seen for the neutral conjugate M+1 ion in aqueous ethanol with 20 mM concentrations of the free resin and amino acids. A larger conjugate peak was observed with addition of tertiary amine as a mild basic catalyst, and the intensity of the conjugate peak exceeded that of the precursors upon replacement of the ethanol with benzene. Resin acids accumulate in the plasma membrane, a non-aqueous environment apparently conducive to conjugation of 7-oxodehydroabietic acid with lysine side chains of membrane proteins. The result would be dehydroabietic acid covalently bound to protein, which could lead to interaction with immune cells having resin acid specificity. The haptenation mechanism presented may be involved in allergic contact dermatitis and occupational asthma observed from exposure to resin acid

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[1]Authored by P.A. Smith, C.R. Bowerbank, P.B. Savage, D.B. Drown, M.L. Lee, W. Alexander, W.W. Jederberg, and K.R. Still

solids and aerosols. As sampling and analytical methods have been previously demonstrated for 7-oxodehydroabietic acid, this compound may be a useful exposure marker with relevance to negative health effects such as occupational asthma.

2.2.2 Introduction

Allergic contact dermatitis[24-26] and occupational asthma[10-12,27-35] have resulted from exposure to rosin via dermal and inhalation routes. The dermal sensitizing capability of pure abietic acid, one of potentially many resin acid constituents of rosin, has been shown to be slight at best,[2] while oxidized resin acid species are stronger dermal sensitizers.[3-5,7,36-39] Further evidence that oxidized resin acids are responsible for the sensitizing capability of rosin lies in the low sensitizing potential of hydrogenated resin acids, which are less susceptible to air oxidation than non-hydrogenated resin acids.[8] Also, several resin acids with epoxide and hydroperoxy groups possess the potential to form reactive chemical species and have been shown to be contact allergens.[5,7,36,38,39] Hypotheses to explain this activity have focused on the reactivity of such compounds (haptens) and their ability to form protein conjugates, which frequently appears to be a requirement for antibody production against low molecular weight compounds.

The oxidized resin acid 7-oxodehydroabietic acid (Figure 2.1.2) has been found by several research groups to be a contact allergen using the guinea pig maximization test (intradermal induction),[3] and also in guinea pigs by Freund's complete adjuvant induction.[4]

Although empirical data show 7-oxodehydroabietic acid to be a dermal sensitizer, no conjugation mechanism has been proposed to explain its biological activity. Hausen *et al.* hypothesized that an electrophilic metabolite of the compound may explain its sensitizing ability.[4] Interestingly, 7-oxodehydroabietic acid has been detected as a common component (at low levels)

in respirable aerosols collected during soldering with rosin flux.[9,40] Thus, in addition to its known dermal sensitizing capability, it may also play a role in occupational asthma resulting from inhalation of rosin-derived aerosol from soldering with rosin flux.

After examination of potential chemistry that may lead to covalent adduct formation of 7-oxodehydroabietic acid, it was noted that the benzylic carbonyl group is predicted to be reactive with amines. A Schiff base, or imine is formed by reaction of an amine and an aldehyde or ketone. The benzylic position of the carbonyl group would tend to increase the electrophilic nature of the carbonyl carbon, which increases the tendency of the compound to form a Schiff base with an amine (Figure 2.2.1). Experiments were carried out *in vitro* to conjugate the oxidized resin acid to the free amino acid L-lysine. Evidence for the conjugate was sought using fast atom bombardment mass spectrometry (FAB/MS).

2.2.3 Materials and Methods

2.2.3.1 Resin Acid/Amino Acid Conjugation

L-(+)-lysine hydrate (99% purity, Acros, Pittsburgh, PA) and 7-oxodehydroabietic acid (95%, Helix Biotech, Richmond, BC, Canada) were dissolved in ethanol (95%, Midwest Grain Products, Atchison, KS), both at 20 mM. The mixture was stirred gently overnight before analysis. Following initial FAB/MS analysis, benzene (Fisher Scientific, Pittsburgh, PA; HPLC grade) was added to the same mixture to form an azeotrope with water present in the system. The resulting azeotrope was boiled at about 60 °C, with benzene added occasionally as ethanol/benzene and residual water boiled off. This proceeded until the boiling point of the mixture approached that of benzene, indicating that the ethanol and water had been removed. Following FAB/MS analysis of the benzene mixture, the benzene was evaporated off and ethanol (100% purity, McCormick Distilling, Weston, MO) was

then added along with 20 mM of N,N-diisopropylethylamine (99% purity, Aldrich, St. Louis, MO) as a weak basic catalyst. Following FAB/MS analysis of the mixture, ethanol/benzene was again boiled off. Benzene was added to replace the lost azeotropic mixture until the mixture again boiled near the boiling point of benzene. Analysis of the resulting benzene mixture with FAB/MS was repeated.

2.2.3.2 FAB/MS Method

A JEOL model JMS SX102A double sector mass spectrometer was used for FAB/MS analyses. A direct insertion probe was used with xenon gas fast atom bombardment ionization.

2.2.4 Results and Discussion

Analysis by FAB/MS of the original experiment in ethanol produced ion signal for the diprotic resin acid-amino acid conjugate at 443 *m/z* $(M+H)^+$, although the intensity of the peak was low relative to the intensity of the $(M+H)^+$ peak for 7-oxodehydroabietic acid. It was felt that the exclusion of water from the reaction would lead to a larger peak for the conjugate, but the first benzene experiment showed little improvement. Base catalysis with the tertiary amine compound N,N-diisopropylethylamine in ethanol increased the relative abundance of the conjugate $(M+H)^+$ peak. When ethanol was replaced with benzene in the amine catalyzed experiment, the conjugate $(M+H)^+$ ion became the base peak. The spectrum of the final experiment is given in Figure 2.2.2.

2.2.4.1 Immunogenicity of Resin Acids

Work by Cullen *et al.* showed that unconjugated rosin compounds failed to elicit an immune response when administered intraperitoneally to mice along with adjuvant, whereas rosin conjugated

to bovine serum albumin via the carboxylic acid functional group of the resin acids present in the rosin led to production of antibodies against the conjugates. These antibodies demonstrated affinity for un-conjugated resin acids as well.[41] Thus haptenation is apparently important in immune system recognition of resin acid compounds. The importance of haptenation in eliciting an immune response to resin acids correlates well with the literature identifying peroxy resin acids as potent dermal sensitizers. Protein conjugation of such compounds is easily visualized via a radical or epoxide mechanism.[5,7,36,38,39]

Contact urticaria (an immediate type skin reaction mediated by pre-formed antibodies) has been found with exposure to rosin.[43] What has been described as occupational asthma from inhalation of rosin air contaminants often appears to include an immediate response,[32,35] which could be explained by binding of pre-formed IgE antibody to resin acids, leading to release of inflammatory substances from basophils and mast cells.[44] It should be noted however that circulating antibodies to colophony have yet to be observed in patients with apparent pulmonary sensitization from exposure to resin acid aerosol. Dermal prick tests with colophony in solution[10] and with colophony conjugated to human serum albumin[35] produced no reactions in apparently sensitized electronics workers. Radioallergosorbent (RAST) tests of affected workers were also negative using colophony/protein conjugates.[35] The author of both of these studies states that "no evidence has been found that IgE antibodies are present, but the search has not been very thorough."[35]

As previously outlined by Burge,[35] aspects of the observed pulmonary hypersensitivity are consistent with preformed IgE antibodies in afflicted patients. In an individual sensitized to unheated colophony aerosol, when solid colophony was crushed with a hammer and aerosolized by passing back and forth between two trays for 15 minutes, a strong immediate asthmatic reaction

occurred.[11] During bronchial provocation testing of solderers with asthma attributed to rosin aerosol, only three breaths of "colophony fumes" produced by heating rosin to 350 °C were needed to produce a strong asthmatic response in one of the workers.[35] The asthma produced in solderers of another study required a mean of 8 years exposure before sensitization developed.[32] To summarize the evidence for pre-formed antibodies as the cause of asthma from exposure to resin acid aerosol, 1) only a fraction of the exposed workers develop the symptoms;[35] 2) in some of the sensitized workers, only a very small dose is needed to produce the asthmatic response;[11,35] 3) a latent period with no signs of sensitization is usually needed to produce asthma from rosin derived aerosol;[32,35] and 4) the proportion of atopic individuals (with elevated IgE levels compared to controls) was higher in an electronics factory population of workers with sensitization compared to those who were not sensitized.[35]

Allergic contact dermatitis to rosin is generally regarded as a delayed (cell-mediated) type of allergic response, where T-cell clones with specificity towards the eliciting agent infiltrate exposed tissues and mediate an inflammatory response which may take hours to develop.[44] In cases of both delayed and immediate hypersensitivity to rosin constituents, haptenation of resin acid compounds is probably needed for immune system recognition of the relatively small resin acid compounds (about 300 daltons).

2.2.4.2 Haptenation of 7-Oxodehydroabietic Acid Via Imine Formation with L-Lysine

The most significant limitation of this study is that the haptenation model was tested *in vitro*. The laboratory conditions used are, however, similar to those likely to be encountered in nonaqueous cellular environments such as the plasma membrane.

Examining Schiff base (or imine) formation as a haptenation mechanism, Dupuis and

Benezra postulated it to be the likely mechanism by which aldehydes combine with protein carriers.[44] The mechanism by which Schiff bases are formed involves nucleophilic attack of an amine on a ketone or aldehyde. Proton exchange is followed by elimination of water (Figure 2.2.1). The requirement for a nucleophilic amine and subsequent proton exchange dictates a moderate pH range in which the reaction will occur: sufficiently basic that the amine will be somewhat unprotonated and thus nucleophilic, yet allowing proton exchange and elimination of water.[45] A tertiary amine catalyst was chosen in the current study, since such a compound can participate in proton exchange, but will not form a Schiff base. In an intact organism, various biological compounds could catalytically provide and accept protons in a similar fashion.

Imines formed from benzylic ketones (such as the ketone in 7-oxodehydroabietic acid) are expected to be more stable than imines formed from nonbenzylic ketones, and a nonaqueous environment such as the plasma membrane matrix will further enhance the stability of the resulting imine.[46] Benezra *et al.* indicate that haptenation in allergic contact dermatitis predominantly occurs with membrane proteins.[47] The amphipathic nature of resin acid compounds dictates that the lipid bilayer is the likely location for cellular accumulation of resin acids, and several papers discuss membrane effects of resin acids.[48,49] Villalain found that abietic acid (which differs from dehydroabietic acid by one double bond) was accumulated in a synthetic plasma membrane system composed of egg yolk phosphatidylcholine.[50]

Schiff base formation is not unheard of in biochemistry. One example of an enzymatic reaction with Schiff base formation is cleavage of fructose 1,6-bisphosphate by class I aldolase, yielding dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.[51] In that reaction a lysine residue exists at the active site and formation of the Schiff base between the ketone group of fructose 1,6-bisphosphate and the NH_2 R-group of the lysine residue is assisted by a serine residue near the

active site acting as a proton acceptor and donor.

Finally, formation of the 7-oxodehydroabietic acid/protein conjugate will yield a conjugate potentially capable of producing antibodies against dehydroabietic acid (Figure 2.1.2) and perhaps other resin acids as well. Dehydroabietic acid is commonly found in many rosin preparations, and antibodies or T-cell clones produced against it could lead to allergic reactions upon subsequent exposure to virtually any rosin product. Interestingly, 7-oxodehydroabietic acid has been observed in aerosol produced from heating rosin core solder,[40] and dehydroabietic acid was the major constituent of aerosol produced upon heating of a variety of rosin core solder fluxes of varying composition, especially in the presence of lead/tin solder.[9,40]

Previous work showing 7-oxodehydroabietic acid to be a dermal sensitizer[3,4] and its presence in aerosol derived from rosin core solder[40] raise suspicions about its role in occupational asthma observed in solderers using rosin flux.[10,27-35] Our data showing that this compound can, without further metabolic activation, bind covalently to a common amino acid provide mechanistic confirmation of its sensitizing potential. Of practical importance to the industrial hygiene profession is the possibility that this compound is a potential exposure marker with apparent relevance to negative health effects such as occupational asthma. Also, sampling and analysis methods for 7-oxodehydroabietic acid have already been demonstrated.[40]

2.2.5 Conclusion

A resin acid/protein haptenation mechanism is presented for an oxidized resin acid found in aerosol produced during soldering with rosin flux, and also found in rosin solids. The compound, 7-oxodehydroabietic acid, has been previously shown to be a dermal sensitizer, although it has no apparent radical haptenation mechanism. Evidence for a conjugate of L-lysine to 7-

oxodehydroabietic acid was obtained using fast atom bombardment/mass spectrometry, and the greatest relative abundance for the conjugate ion was seen under non-aqueous conditions using a tertiary amine catalyst. The proposed haptenation mechanism involves formation of a Schiff base covalent bond between C-7 of the resin acid and a free amino group of lysine. As resin acids accumulate in non-aqueous cellular membranes, haptenation of 7-oxodehydroabietic acid in that environment would result in dehydroabietic acid covalently bound to lysine residues of membrane protein at the C-7 position. This could lead to antibody production against dehydroabietic acid, a resin acid found as a major fraction of aerosol derived from heating rosin flux and in samples of solid rosin. In addition to a likely role in dermal sensitization, haptenation of 7-oxodehydroabietic acid may be involved in occupational asthma from chronic inhalation of resin acid aerosol during soldering with rosin flux.

2.2.6 Acknowledgments

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2.3 Airborne Aldehydes From Heating Rosin Core Solder and Liquid Rosin Flux to Soldering Temperatures[1]

2.3.1 Abstract

Gas phase aldehydes produced from heating rosin core solder and liquid rosin flux to temperatures commonly used in soldering were trapped on sampling tubes containing XAD-2 resin coated with the derivatizing agent 2-hydroxymethylpiperidine. Analysis of the resulting oxazolidine derivatives was performed using gas chromatography/mass spectrometry. The observed aldehyde derivatives included formaldehyde, acetaldehyde, propionaldehyde, acrolein, isobutyraldehyde, butyraldehyde, isovaleraldehyde, valeraldehyde, furfural, hexanal, cyclohexane carboxaldehyde, and other unidentified compounds likely to be aldehyde isomers. Formaldehyde, acetaldehyde, and benzaldehyde were detected in blank samples. By comparison with an internal standard, a sample produced by drawing air with contaminants derived by heating rosin core solder through a sampling tube contained levels of formaldehyde and acetaldehyde much greater than seen in sampling tube blanks. Benzaldehyde was not shown to be present at a significantly greater level in samples from heating rosin core solder than in blanks prepared using the same analysis protocol. The use of NIOSH method 2539 extraction procedures produced blanks with levels of formaldehyde significantly lower than with a modified extraction method (methylene chloride, no sonication). The modified extraction method produced significantly lower benzaldehyde levels in blanks compared to the NIOSH extraction method using toluene and sonication of sampling sorbent tubes.

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2.3.2 Introduction

The possibility that formaldehyde is a carcinogen has gained acceptance,[52] and other short chain aldehydes such as acetaldehyde are also being scrutinized to examine their potential for similar activity.[53] Aldehydes are potentially acutely toxic *via* inhalation exposure, with the inhalation toxicity of aldehydes of up to 5 carbons shown somewhat to correlate with decreased molecular weight.[54] The unsaturated aldehydes acrolein (2-propenal) and crotonaldehyde (2-butenal) examined in the same study were found to posses increased acute toxicity in three species (in terms of mean fatal exposure concentrations). Additionally, short chain aldehydes are recognized as irritating at low concentrations.[55] Structures of several aldehydes are given in Figure 2.3.1.

In previous studies to examine aldehydes produced by heating rosin, Drugov and Murav'eva used gas chromatography (GC) with flame ionization detection (FID) to observe gaseous contaminants produced when a soldering iron was immersed in rosin.[56] They identified formaldehyde specifically, and other aldehydes ranging in size from 5 to 8 carbon atoms non-specifically. Silica gel was used as the sampling medium in their work.

Burge *et al.* sampled in an electronics factory where rosin core solder was heated during manufacture of rosin core solder to temperatures similar to those encountered during soldering (300-400 °C) and obtained evidence that aldehydes were present in the air of the factory.[10] The sampling method in their study was non-specific, and used an impinger with a 0.05% solution of 3-methyl-2-benzolthiazolone hydrochloride. This solution forms a blue cationic dye upon reaction with aliphatic aldehydes in an acidic solution, and subsequent spectrophotometric analysis for aldehydes was performed.

Guenier *et al.*[57] showed formaldehyde, acetaldehyde, propionaldehyde, and acrolein to be present as gaseous contaminants produced by heating rosin in an oven at 250 and 400 °C. These

researchers used silica gel coated with 2,4-dinitrophenylhydrazine to trap the aldehydes, with the resulting 2,4-dinitrophenylhydrazones analyzed by gas chromatography with FID detection.

The most recent attempt to identify aldehydes produced by heating rosin flux involved quantitative measurements by Pengelly *et al.*[58] They heated rosin core solder of various types in the laboratory and captured the resulting airborne aldehydes with an impinger. The impinger derivatizing agent was 3-methyl-2-benzothiazolone hydrazone, and following trapping/color development, the impinger solution was subjected to thin layer chromatography (TLC). Quantitative estimates for formaldehyde and acetaldehyde derivatives were obtained by densitometry of the resulting TLC spots for each compound. The acetaldehyde derivative was found to be present at slightly higher levels than that of formaldehyde at temperatures between 300 and 370 °C, while the formaldehyde derivative predominated between 370 and 450 °C. There was no definitive identification of other airborne aldehydes produced from heating rosin core solder.

The continued interest in reactive aldehyde compounds with a range of physical properties has produced a number of sampling and analytical methods to trap, separate, and identify the various aldehyde species present in samples of various types. Sampling and analytical methods for occupational exposures to aldehydes have been produced by the National Institute for Occupational Safety and Health (NIOSH), and the U.S. Department of Labor, Occupational Safety and Health Administration (OSHA). Most frequently used aldehyde workplace sampling methods take advantage of the reactivity of aldehydes to trap them on a sorbent coated with a derivatizing agent. Two commonly used derivatizing agents are 2-hydroxymethylpiperidine (2-HMP) and 2,4-dinitrophenylhydrazine (DNPH). As examples, the NIOSH sampling and analytical method 2539 for aldehyde screening[59] and the OSHA method 68 for sampling and analysis of acetaldehyde[60] use XAD-2 resin coated with 2-HMP to trap and derivatize aldehydes. Methods using DNPH include

OSHA method 52 for sampling and analysis of acrolein and/or formaldehyde.[61]

The methods used in this study were based on NIOSH method 2539. As the derivatizing agent in method 2539, 2-HMP does not derivatize ketones (as does DNPH), and as explained below, the 2-HMP aldehyde derivatives are well suited for mass spectrometric detection with high sensitivity. Following derivatization on the tube medium, desorption is carried out with toluene, and the oxazolidine derivatives of specific aldehydes may be separated, identified, and quantified by gas chromatography with a suitable detector.

A mass spectrometric detector offers considerable advantages for this work, as the mass spectra for all oxazolidine aldehyde derivatives examined (except furfural oxazolidine) exhibit strong ion current for the *m/z* 126 ion. For the non-aromatic aldehyde oxazolidine derivatives studied here, this ion provides the base peak in the resulting mass spectra. The oxazolidine derivatives are bicyclic compounds, which upon loss of aldehyde R-groups show the stable 126 *m/z* fragment. Sensitivity is increased (compared to the total ion current chromatogram) by extracting the 126 *m/z* ion current to produce an extracted ion chromatogram.

The derivatization scheme is outlined in Figure 2.3.2. For aldehyde oxazolidine derivatives (except formaldehyde), a stereocenter is located at the ring carbon where the R-group is attached. Thus, two diastereomers are potentially possible for each aldehyde oxazolidine.

2.3.3 Materials and Methods

2.3.3.1 Laboratory generation of rosin-derived aldehydes

An apparatus was constructed to trap rosin pyrolysis products. Figure 2.3.3 shows the design of the apparatus. Dried air was drawn past the soldering iron tip, and then through a glass region suitable for use as a cold trap. The air was pulled through the apparatus at 150 mL per minute using

a low flow pump (Pocket Pump, SKC, Eighty Four, PA) and samples were trapped either by the cold trap (using liquid N_2 to cool the trap), followed by sampling upon warming of the trap to room temperature, or by direct sampling (without cooling). In both cases, an XAD-2 resin tube coated with 2-hydroxymethylpiperidine (SKC, catalog #226-27) was used to sample the air drawn through the pump. Several types of samples were made by heating liquid rosin flux (Kester Solder, Des Plaines, IL, #1544; or, Alphametals, Alpha 100R rosin flux) which was slowly injected using a microprocessor controlled syringe pump (Harvard Apparatus, Harvard '22', Holliston, MA) onto a heated soldering iron placed in the sampling apparatus as shown in Figure 2.3.3. Several types of rosin core solder (Kester Solder, Des Plaines, IL, 60/40 Pb/Sn "44"; or, Chemtronics, Kennesaw, GA, 60/40 Pb/Sn rosin core solder) were also heated in the experiments. Both rosin core solder and liquid flux were introduced through ports on the sampling apparatus through rubber septa, as shown in Figure 2.3.3. The soldering iron (Cooper Tools, Weller EC1201, Apex, NC) was temperature-controlled (Cooper Tools, Weller EC2002M, Apex, NC). Samples were made at typical soldering temperatures of both 350 and 400 °C.

2.3.3.2 Extraction of sampling tubes

NIOSH method 2539 was used for initial extraction. A summary of our adaptation follows: sampling tubes were scored and opened, and adsorbent material was removed into a 5 mL vial with a polytetrafluoroethylene (PTFE) lined lid. A 1.0 mL aliquot of toluene (Mallinckrodt, Paris, KY; spectrophotometric grade) was then added, and the vial was placed in a sonicator for 1 h.[59] It was noted that following sonication, the vial and contents were quite warm, and endogenous production of aldehydes from toluene decomposition was possible. An alternative extraction method replaced toluene with methylene chloride followed by gentle mixing for 20-30 min and no sonication.

2.3.3.3 GC/MS methodology

A Finnigan MAT GCQ GC/MS instrument equipped with a 30 m x 25 μm i.d. (0.25 μm d_f) DB-5 MS capillary column (J&W Scientific, Folsom, CA) was used for GC/MS analyses. Helium carrier gas, with a constant velocity flow rate of 40 cm/s was used. For all samples, the column temperature was maintained at 40 °C for 1.0 min and then increased 10 °C/min to 265 °C, and then 30 °C/min to 300 °C.

Samples were analyzed using the splitless injection mode. The injector temperature was kept at 225 °C, and the GC/MS transfer line was maintained at 275 °C. Electron ionization (70 eV) was used for all samples with an ion source temperature of 150 °C. The scan rate was 0.5 s/scan and mass spectra were collected over the mass-to-charge range of 20-400 *m/z*.

2.3.3.4 Identification of aldehyde derivatives and quantitative comparisons

Identification of oxazolidine aldehyde derivatives was accomplished by comparison of retention times and mass spectra with standards for the aldehyde derivatives. Standards were produced by direct spiking of the corresponding aldehyde onto a small amount of the XAD-2 resin coated with derivatizing agent placed in a 5 mL vial. Desorption was as described previously using toluene, although standards so produced were not subjected to sonication. Aldehyde standards were purchased (Aldrich Chemicals, Milwaukee, WI) with the following purities: formaldehyde (37%, 10-15% methanol), acetaldehyde (99%), propionaldehyde (97%), acrolein (90%), isobutyraldehyde (99%), butyraldehyde (99.5%), isovaleraldehyde (97%), valeraldehyde (97%), furfural (99%), hexanal (98%), and cyclohexane carboxaldehyde (98%). Benzaldehyde (purity not stated) was purchased from Sigma (St. Louis, MO).

In several samples, *d*-camphor (Baker Grade, J.T. Baker Chemical Company, Phillipsburg,

NJ) was used as an internal standard, allowing a quantitative comparison of aldehydes from several sample types. Comparison of aldehyde levels in blanks made by the standard NIOSH method with sonication, to those made by methylene chloride extraction without sonication was done with the internal standard at the same concentration in both sample types. Use of the internal standard also allowed a comparison of aldehyde concentrations between a rosin-core solder sample and blanks containing the same concentration of internal standard. Three replicates of blanks were prepared using the NIOSH extraction methodology (toluene extraction with sonication). These were compared with three replicates prepared by methylene chloride extraction and no sonication, using two-tailed *t*-tests (equal and unknown variance). Individual replicates were provided by the integrated peak values of the oxazolidine derivatives of formaldehyde, acetaldehyde, benzaldehyde, and an unknown compound (probable aldehyde) eluting near benzaldehyde with an apparent 7-carbon structure and one unit of unsaturation (by examination of the peak's mass spectrum). Confidence intervals were also calculated for the resulting means of the integrated peaks for both types of blanks. In samples with internal standard added, the 152 *m/z* extracted ion current was used in addition to that of 126 *m/z* to produce chromatograms, as 152 *m/z* represents the molecular ion of *d*-camphor.

2.3.4 Results

2.3.4.1 Gas chromatograph/mass spectrometry of laboratory generated samples

Example GC/MS chromatograms from extracted 126 *m/z* ion current (Figure 2.3.4) or extracted 126 *m/z* + 152 *m/z* ion current (Figures 2.3.5 and 2.3.6) from analysis of laboratory generated samples are shown. Aldehyde oxazolidines were detected in all types of samples created with rosin core solder and liquid rosin flux and included formaldehyde, acetaldehyde (both

derivative diastereomers were observed), propionaldehyde, acrolein, butyraldehyde, isobutyraldehyde, valeraldehyde, isovaleraldehyde, 2-methylbutanal, furfural, hexanal, cyclohexanecarboxaldehyde, and benzaldehyde. Figure 2.3.5 shows a chromatogram for a laboratory blank, with formaldehyde, acetaldehyde and benzaldehyde visible. Furfural was not seen in any of the 126 *m/z* extracted ion current chromatograms, but was observed in various samples from heating rosin at both temperatures by examining 192 *m/z* extracted ion chromatograms.

Unlabeled peaks in the figures are likely aldehyde oxazolidine derivatives with unknown molecular conformations. Some unidentified probable aldehyde derivatives (such as peak *k* in Figure 2.3.6 chromatograms) were present in sufficient abundances to estimate the *m/z* ratio of the derivative molecular ion, allowing an estimate of the molecular weight of the parent aldehyde. A weak molecular ion current was characteristic for derivatives of non-aromatic aldehydes, and this method was not useful for estimating the molecular ion *m/z* ratios for small peaks. A rather large number of 4-7 carbon aldehyde isomers are possible, and the unlabeled chromatogram peaks are probably in this range as they elute between isobutyraldehyde and a point near benzaldehyde. Aldehydes of higher molecular weight were not observed. This may be due to the inverse relationship between molecular weight and volatility, and it is thus possible that such compounds (if produced during heating of the rosin flux) would be less likely to be trapped as vapor phase contaminants.

Some differences were seen with respect to peak size when comparing samples made with different types of flux and different temperatures, but essentially all of the aldehydes shown in Figure 2.3.4 were also observed in the other samples. The use of direct sampling (without cold trapping) gave qualitatively richer 126 *m/z* extracted ion chromatograms than did cryogenic trapping followed by trapping on the sampling medium. The increase in formaldehyde relative to

acetaldehyde at higher temperatures as noted by Foster *et al.*[58] was not observed in our results.

The ratio of aldehyde oxazolidine integrated peaks to that of the *d*-camphor internal standard showed that between blanks prepared according to the different methods used, there was no difference (at $p=0.10$) between acetaldehyde derivative levels from both types of blanks. Formaldehyde derivative levels were higher in the blanks prepared using methylene chloride extraction ($p<0.005$), benzaldehyde derivative levels were higher with toluene extraction and sonication ($p<0.005$), and levels of the unknown probable aldehyde derivative eluting near benzene were greater in the toluene extracted blanks ($p<0.05$).

When comparing the sample produced by heating rosin core solder, extracted with methylene chloride (no sonication), and internal standard added, both formaldehyde and acetaldehyde levels were well above those observed in the blanks produced by the same method, but levels of benzaldehyde and the unknown likely aldehyde derivatives were within the 95% confidence intervals calculated for the blank samples prepared with the same method. Table 2.3.1 summarizes the data for these experiments.

In samples produced by heating both liquid rosin flux and rosin core solder, monoterpene compounds such as α-pinene were also seen in the resulting total ion current chromatograms as vapor-phase contaminants. Such 10 carbon terpene constituents emitted in the vapor phase appeared to be quite diverse, and further studies are planned to more fully characterize these compounds.

2.3.5 Discussion

2.3.5.1 GC/MS analyses

The observation of both possible diastereomers of the acetaldehyde derivative agrees with

the discussion found in OSHA method 68 documentation.[60] Our results confirm those of Guenier *et al.*[57] who observed formaldehyde, acetaldehyde, propionaldehyde, and acrolein as air contaminants produced by heating rosin. The increased sensitivity of the mass spectrometric detector over FID used by Guenier *et al.*[57] allowed the confirmation of several additional aldehydes as oxazolidine derivatives. Interestingly, acetaldehyde and propionaldehyde signals were greater than those for formaldehyde in our rosin-derived samples. Previous American Conference of Governmental Industrial Hygienists Threshold Limit Values for exposure to rosin core solder pyrolysis products were at one time expressed in terms of formaldehyde.[62] In fact, formaldehyde exposure has been widely used as a surrogate when measuring personal exposure to airborne rosin core solder pyrolysis products,[10,63] although from our results, other aldehyde compounds such as acetaldehyde and propionaldehyde may be equally or more significant in terms of amount of airborne aldehydes produced from heating rosin flux.

The presence of monoterpene compounds such as α-pinene in the gas phase contaminants produced by heating rosin flux is in agreement with the observations of Pengelly *et al.*[58] Also, previous work has identified diterpene resin acid compounds in aerosol produced by heating rosin flux during soldering.[9,50,58] One of those identified as a commonly found resin acid in workplace aerosol samples taken during soldering (7-oxodehydroabietic acid)[50] is a known dermal sensitizer.[3,4] Pulmonary exposure to such a sensitizing compound could account for an unknown portion of the observed pulmonary sensitization which is linked with exposure to rosin aerosol produced either from heated rosin,[36] or if the compound is present (as may be likely due to our observation that it is commonly found in solid rosin) from exposure to unheated aerosols containing rosin.[11,12]

That workers exposed to unheated rosin-derived aerosols could develop occupational asthma indicates that pulmonary exposure to resin acids alone is probably capable of producing the disease. However, the presence of aldehyde compounds as air contaminants produced by heating rosin flux complicates the situation somewhat, as formaldehyde has been shown to potentiate the production of asthma in guinea pigs exposed to ovalbumin.[64] The work described in a previous paper[9] indicates that at soldering temperatures, most of the rosin flux heated to vaporization rapidly recondenses to the solid state and a minor portion is potentially lost to thermal decomposition products with non-aerosol characteristics. The relative contribution of such non-aerosol contaminants (such as the aldehyde compounds studied in this instance) to worker health effects is unknown at this time.

2.3.6 Conclusions

Gas phase aldehydes produced from heating rosin core solder and liquid rosin flux were trapped on sampling tubes containing XAD-2 resin coated with the derivatizing agent 2-hydroxymethylpiperidine. Analysis of the resulting oxazolidine derivatives was performed using gas chromatography/mass spectrometry. The aldehyde derivatives observed included formaldehyde, acetaldehyde, propionaldehyde, acrolein, isobutyraldehyde, butyraldehyde, isovaleraldehyde, valeraldehyde, furfural, hexanal, cyclohexane carboxaldehyde, and several unidentified compounds likely to be aldehyde isomers. Aldehyde oxazolidines were detected in samples created with several types of liquid rosin flux and rosin core solder.

Laboratory blanks showed derivatives of formaldehyde, acetaldehyde and benzaldehyde. The ratio of aldehyde oxazolidine integrated peaks to that of *d*-camphor internal standard showed that between replicate blanks prepared according to the standard NIOSH method 2359, and the same

method using methylene chloride (no sonication) there was no difference between acetaldehyde derivative levels from both types of blanks. Formaldehyde derivative levels were higher in the blanks prepared using the methylene chloride extraction, benzaldehyde derivative levels were higher with toluene extraction and sonication, and levels of the unknown probable aldehyde derivative eluting near benzene were greater in the toluene extracted blanks. When comparing the sample produced by heating rosin core solder, extracting with methylene chloride (no sonication), and addition of internal standard, both formaldehyde and acetaldehyde levels were well above those observed in the blanks produced by the same method, but levels of benzaldehyde and the unknown likely aldehyde derivatives were within the 95% confidence intervals calculated for the blank samples prepared with the same method.

2.3.7 Acknowledgments

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2.4 Metabolism of Dehydroabietic Acid by Rat Liver Enzyme Preparation[1]

2.4.1 Abstract

An *in vitro* system was used to examine mammalian metabolites of dehydroabietic acid, a common resin acid component of rosin and a commonly observed resin acid component of aerosol produced from heating rosin flux. Metabolites were produced using rat liver S9 fraction enzymes in a phosphate buffer. Dehydroabietic acid, $NADP^+$ cofactor, and glucose-6-phosphate reducing equivalents were added to the system followed by incubation at 37 °C. After incubation the system was extracted and resin acids present in the extract were methylated for analysis by gas chromatography/mass spectrometry. The resin acid metabolites observed following a 1 hour incubation period experiments were abieta-8,11,13,15-tetraen-18-oic acid, and 15-hydroxy-dehydroabietic acid.

2.4.2 Introduction

Dehydroabietic acid (DHAA) is a resin acid constituent of rosin, which possesses a single aromatic ring. The aromatic ring is likely the cause of this compound's stability relative to abietadienoic resin acids such as abietic acid (Figure 2.1.2). Numerous references provide confirmation of the environmental stability of DHAA relative to abietic acid, another common resin acid constituent of rosin.[17,23,50] Previous papers have shown DHAA to be consistently present in aerosol derived from heated rosin flux,[9,65] while the presence of abietic acid in such aerosol was sporadic.[9,65] In samples collected during light soldering, abietic acid was observed

[1]Authored by P.A. Smith, C.R. Bowerbank, D.B. Drown, and M.L. Lee

at lower levels relative to the proportion of these two resin acids in the unheated parent materials.[9,65]

The aerosol produced during use of rosin core solder has been examined for the presence of oxidized resin acids, regarded as sensitizers.[9] The presence of such compounds in the aerosol may explain the pulmonary sensitization observed in some individuals chronically exposed to rosin core soldering.[1] Sensitizing resin acid compounds such as 7-oxodehydroabietic acid[4,6] (which was consistently observed in samples collected in workplaces where rosin core solder was used) could account for the observed sensitization. Alternatively, non-sensitizing resin acids which predominate in the aerosol produced by soldering with rosin core solder could possibly be metabolized, leading to exposure to other compounds which have increased sensitizing potential.

Owing to the dominant nature of dehydroabietic acid in workplace air samples (relative to other resin acids) where soldering with rosin core solder was performed, this compound was selected for *in vitro* metabolism studies. Rat liver S9 fraction derived from Aroclor 1254 induced animals, and prepared according to Ames *et al.*[66] was used to produce dehydroabietic acid metabolites *in vitro*. Dehydroabietic acid metabolites have previously been produced *in vivo* and separated and identified, following oral administration of the compound to rabbits.[67-69] Comparative information regarding tissue-specific metabolism of dehydroabietic acid in another species was provided by the current study.

Resin acid compounds present in the metabolism system were analyzed by gas chromatography/mass spectrometry (GC/MS) as methyl esters following incubation of dehydroabietic acid with the microsomal enzyme preparation.

2.4.3 Materials and Methods

2.4.3.1 Metabolism System

The metabolism system used was based on that proposed by Ames *et al.*[66] for use in *Salmonella* mutagenicity assays. The metabolism system was constituted in a phosphate buffer (pH 7.4) which had the following concentrations of components per mL: $MgCl_2$ (8 μmoles, Mallinckrodt, Paris, KY; analytical reagent grade), KCl (33 μmoles, Mallinckrodt, analytical reagent grade), glucose-6-phosphate (5 μmoles, Sigma, St. Louis, MO; 99%), NADP (4 μmoles, Sigma, 99%), and sodium phosphate (100 μmoles, Mallinckrodt, analytical reagent grade). Rat liver S9 mix, prepared according to Ames *et al.*[66] was added at the rate of 0.1 mL per 1 mL of overall buffer mix.

Dehydroabietic acid was added to the test system as follows: dehydroabietic acid (>99%, Helix Biotech, Richmond, BC, Canada) was brought up in ethanol (Fisher, Pittsburgh, PA; HPLC grade) and diluted to provide a 1.4% ethanol (v/v) solution with Na_2CO_3 (0.1 M, Mallinckrodt, analytical reagent grade) in distilled H_2O providing a final dehydroabietic acid concentration of 0.143 mg/mL. The amount of dehydroabietic acid added per mL of metabolism test system was 50 μg and the final concentration of ethanol in the test system was 0.5% (v/v).

Each metabolism experiment consisted of 1 mL total volume, including buffer/salts, $NADP^+$, glucose-6-phosphate, S9, and dehydroabietic acid. Also, in addition to experiments carried out with active S9, dehydroabietic acid was also added to a system identical in all respects, except for the use of S9 preparation which had been inactivated by boiling for 10 minutes.

2.4.3.2 Extraction and Solvent Transfer

Extraction was carried out with direct acidification by a saturated aqueous solution of KH_2PO_4 (Mallinckrodt, analytical reagent grade) and methylene chloride extraction. The methylene

chloride fraction was passed over Na_2SO_4 (Fisher, A.C.S. anhydrous) to remove water and was subsequently evaporated to dryness at 70 °C under a gentle stream of N_2. Dimethylformamide (DMF, 0.5 mL, Mallinckrodt, analytical reagent grade) was used as a solvent for GC/MS analyses.

2.4.3.3 Sample Derivatization and Analysis

Carboxylic acid groups present in the samples were esterified by addition of a methyl group to allow chromatographic separation of resin acid compounds, as previously described.[9]

A Finnigan MAT GCQ GC/MS instrument equipped with a 30 m DB-5 MS capillary column (J&W Scientific, Folsom, CA) was used with splitless injection. The column inside diameter was 0.25 mm, and the stationary phase thickness was 0.25 μm. The carrier gas was helium, with a constant velocity flow rate of 40 cm/sec. For all samples, column temperature was maintained at 140 °C for 1.0 minute and then increased 40 °C/min to 200 °C, and then 5 °C/min up to 300 °C. The injector temperature was kept at 250 °C. The GC/MS transfer line was maintained at 275 °C. Electron ionization (70 eV) was used for all samples, with an ion source temperature of 150 °C. Scan rate was 0.5 seconds/scan and mass spectra were collected over the range 50-650 *m/z*.

Dehydroabietic acid (>99%), and 7-oxodehydroabietic acid (95%) were purchased for reference standards (Helix Biotech). An authentic sample of 15-hydroxydehydroabietic acid methyl ester was synthesized using the methodology of Shao *et al.*[7] with the structure confirmed using infrared spectrometry, ^1H nuclear magnetic resonance, and mass spectrometric methods.

Individual resin acid compounds were identified as the methyl esters by relative retention times (dehydroabietic acid = 1.00) and mass spectra comparison to either standards or reference data.[7,67]

2.4.4 Results

In addition to dehydroabietic acid, two resin acid compounds were observed in extracts from metabolism system solutions following incubation: 15-hydroxydehydroabietic acid, and abieta-8,11,13,15-tetraen-18-oic acid. Only dehydroabietic acid was observed in negative control extracts from metabolism systems using S9 preparation which had been boiled to denature metabolic enzymes. The structures of the two observed metabolites are given in Figure 2.4.1. A GC/MS chromatogram showing the results of a metabolism experiment is provided as Figure 2.4.2.

2.4.5 Discussion

No literature references were found regarding the sensitizing ability of abieta-8,11,13,15-tetraen-18-oic acid. The compound 15-hydroxydehydroabietic acid has been found to be a sensitizer in the guinea pig maximization test by one research group.[3] Another group, however found this compound to be non-sensitizing using the Freund's complete adjuvant method in a guinea pig model.[5]

The metabolites observed by other researchers in whole animal rabbit oral dosing experiments included 15-hydroxydehydroabietic acid, and abieta-8,11,13,15-tetraen-18-oic acid,[67-69] which were both observed in the current experiments. Additionally, other metabolites were observed in the referenced whole animal experiments. These included 7-oxodehydroabietic acid (in trace amounts),[67,68] several dihyhdroxy-dehydrobietic acid compounds,[69] and another mono hydroxy-dehydroabietic acid compound 16-hydroxydehydroabietic acid.[67-69]

The 15 position of dehydroabietic acid is predictably reactive in a metabolism scheme as a result of the aromatic ring structure one bond removed from this location, and the flanking methyl groups. These features will provide relative stability of a carbocation radical on the benzylic 15

carbon. Such a site on a substrate molecule would interact well with cytochrome p-450 (CYP) enzymes for hydroxylation, which is thought to occur through hydrogen atom abstraction from the substrate (R-H), followed by transfer of CYP bound hydroxy radical to the resulting intermediate alkyl radical (R•) substrate. The hydroxylation of dehydroabietic acid at the 15 position, as well as formation of a double bond across the 15 to 16 carbon bond are both consistent with reactions which could be catalyzed by (CYP) enzymes present (potentially along with other enzymes) in the liver enzyme system used.

As 7-oxodehydroabietic acid was recovered in only trace amounts by other researchers using whole animal metabolic models but was not observed in our experiments using a metabolism system derived from specific tissue, it is possible that this compound may have been formed in the gut during the *in vivo* experiments. Bacterial production of 7-oxodehydroabietic acid has been confirmed by Beillmann and Branlant.[71] The lack of 16-hydroxydehydroabietic acid as a metabolite from the current *in vitro* study is confirmed by a lack of additional peaks with 330 *m/z* molecular ions.

The sensitizing capability of 15-hydroxydehydroabietic acid is equivocal.[3,5] However, further metabolism could produce a hydroperoxy resin acid from this compound. The compound 15-hydroperoxydehydroabietic acid is a known sensitizer[7] which has been prepared in the laboratory as its methyl ester by simply adding H_2O_2 to 15-hydroxydehydroabietic acid methyl ester. Such a pathway could occur for the dehydroabietic acid metabolite in macrophages, which are known to produce H_2O_2.

2.4.6 Conclusions

Dehydroabietic acid has been shown to be a main component of aerosol collected in workplaces where rosin core solder is used in electronics soldering. The possibility exists that metabolism of this resin acid, with low sensitizing potential, may lead to production of one or more resin acids which could be stronger sensitizers. An *in vitro* enzyme system based upon that commonly used to metabolize promutagens in the "Ames" *Salmonella* mutagenicity assay was used to examine mammalian metabolites of dehydroabietic acid. GC/MS analysis showed two metabolites following incubation of the metabolism system and solvent extraction: abieta-8,11,13,15-tetraen-18-oic acid, and 15-hydroxydehydroabietic acid.

This data was confirmatory of resin acid metabolites observed by other researchers who dosed rabbits orally, although several other compounds observed by those researchers were not observed in the current study.

Literature data regarding the sensitizing capacity of 15-hydroxydehydroabietic acid is equivocal, although further biotransformation by macrophagal production of H_2O_2 could lead to production of a sensitizing compound which is a relatively strong sensitizer, 15-hydroperoxydehydroabietic acid.

2.4.7 Acknowledgments

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2.5 Packed Capillary Column Solvating Gas Chromatography of Aldehydes[1]

2.5.1 Abstract

The rapid separation of aldehydes of environmental interest was investigated using packed capillary column solvating gas chromatography (SGC). Fused silica capillary columns (250 μm i.d.) were packed with poly(dicyanoallylsiloxane)-encapsulated 10 μm spherical porous (80 Å) silica particles. Excellent resolution and good peak shapes were achieved for underivatized aldehydes using carbon dioxide as mobile phase. The effects of temperature and pressure on column efficiency and selectivity were studied. Packed capillary SGC gave better resolution of ten standard aldehydes than open tubular gas chromatography using poly(biscyanopropylsiloxane) as stationary phase, and near baseline resolution of these aldehydes was obtained in approximately 60 seconds.

2.5.2 Introduction

Recently, a new separation method, particularly useful for fast separations, called solvating gas chromatography (SGC) was introduced which utilizes compressible, solvating mobile phases.[72-77] The utilization of a solvating mobile phase in packed column gas chromatography (GC) can greatly improve chromatographic performance, including column efficiency and separation speed.[72-73] The applicability of SGC for the analysis of different compound classes has only begun to be examined.[77]

Separation of underivatized aldehydes necessitates the use of polar stationary phases in order to achieve the required selectivity. Low molecular weight aldehydes can differ structurally by only

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[1]Authored by C.R. Bowerbank, Y. Shen, P.A. Smith, D.B. Drown, and M.L. Lee

a double bond, thus the stationary phase must provide a high degree of selectivity in order to successfully resolve these aldehydes. In this study, low molecular weight aldehydes were separated without derivatization using polymer-encapsulated porous silica in packed capillary SGC. We also compared the SGC results to open tubular column GC analysis of the same aldehydes.

2.5.3 Experimental

2.5.3.1 Materials and instrumentation

All aldehyde standards were purchased from Aldrich (Milwaukee, WI, USA). Spherical porous (80 Å) 10 μm silica particles were purchased from Phenomenex (Torrance, CA, USA). The silica particle deactivation polymer, 25% cyanopropyl-substituted polymethylhydrosiloxane, was synthesized in-house. The SGC stationary phase, poly(dicyanoallylsiloxane) [OV-275®], and the open tubular GC column, poly(biscyanopropylsiloxane) [SP-2340], were purchased from Supelco (Bellefonte, PA, USA). Fused silica capillary tubing was purchased from Polymicro Technologies (Phoenix, AZ, USA). Column packing and SGC experiments were performed using the same instrument as described in Reference (100) with the exception that the chart recorder was replaced with a data acquisition system (EZ Chrome version 6.6, Scientific Software, San Ramon, CA, USA) using an SS420 analog-to-digital converter (Scientific Software) which sampled the FID signal at 60 Hz with an IBM-compatible Pentium 166 PC running Windows 95.

2.5.3.2 Preparation of OV-275®-encapsulated particles for SGC

Porous silica (0.20 g of 10 μm, 80 Å) was coated with 0.040 g of 25% cyanopropyl-substituted polymethylhydrosiloxane following a previously described method.[77] The particles were then transferred into a reaction vessel[78] with 0.030 g of OV-275® stationary phase dissolved

in 20 mL of dichloromethane. Argon gas was then bubbled through the mixture at room temperature (~25 °C) until the solvent evaporated. Fused-silica capillary columns (250 μm i.d.) were then packed using a CO_2 slurry method previously described.[79]

2.5.3.3 GC experiments

GC experiments were carried out using manual injection on a Hewlett-Packard 5890 Series II gas chromatograph with FID detection. Helium mobile phase was used at a split ratio of 100:1. Data were acquired using the data acquisition system mentioned above.

2.5.4 Results and discussion

2.5.4.1 SGC of aldehydes

In this study, both saturated and unsaturated low molecular weight (C1-C7) aldehydes were used as test solutes. Because of the structural similarities of these aldehydes, inert and selective stationary phases are needed to provide sufficient resolution. Polymer-encapsulated particles are more inert than conventional bonded phases[80] and cyano groups have been shown to provide selective interaction with double bonds.[81-84] For this study, silica particles were first deactivated with 25% cyanopropyl-substituted polymethylhydrosiloxane and then coated with OV-275®. The deactivation step eliminates many of the strong polar silanol groups on the silica surface, and the OV-275® coating provides a highly polar stationary phase. Figure 2.5.1 shows an SGC separation of various aldehydes using both OV-275®-encapsulated and ODS-bonded particles. The OV-275®-encapsulated particles provided excellent resolution of the aldehydes with good peak shapes and minimal tailing, while the commercial ODS-bonded particles gave tailing peaks and poor selectivity. This suggests that the OV-275®-encapsulated particles are more inert than the ODS-bonded particles and simultaneously provide the desired selectivity.

2.5.4.2 Column Efficiency and Selectivity

One advantage of SGC is its ability to utilize the solvating power of the mobile phase to enhance analysis speed. As the inlet pressure is increased, the solvating power of the mobile phase increases, resulting in lower solute retention and thus an increase in N_t. Increasing the column inlet pressure from 150 to 250 atm at constant temperature led to a decrease in overall column efficiency from 60,000 to 43,000 theoretical plates, but a significant increase in N_t from 181 to 290 plates s^{-1}. Efficiency calculations for both constant temperature and constant pressure conditions are listed in Table 2.5.1a.

Selectivity in SGC can be greatly affected by changing either the temperature or pressure. This is shown in Table 2.5.1b. Increasing the temperature from 70 to 170°C (at constant inlet pressure) resulted in a decrease in the selectivity for caproic aldehyde and heptaldehyde by 0.2. Constant temperature conditions revealed similar results. Even among similar aldehydes, changing the mobile phase properties appears to significantly affect their separation. Using a combination of pressure and temperature, the selectivity can be optimized for the separation of closely related compounds.

The observance of only a slight increase in mobile phase linear velocity with increasing pressure initially seemed unusual. When the temperature was increased from 90 to 170°C at 170 atm column inlet pressure, only a 4% increase in linear velocity occurred. However, this was also observed when using carbon dioxide in supercritical fluid chromatography[85,86]; similar results would be expected under SGC conditions.

2.5.3.3 GC versus SGC

Stationary phases such as SE-54 (94% dimethyl, 5% phenyl, 1% vinyl polysiloxane) are routinely used in GC for a wide range of separations, however, stationary phases with higher polarity and selectivity are required to adequately separate all of the low molecular weight aldehydes studied here. Even when a test mixture was injected at relatively low temperature (40 °C), the SE-54 stationary phase was not able to separate isobutyraldehyde and butyraldehyde, as shown in Figure 2.5.2. The SE-54 stationary phase likewise does not possess enough selectivity to resolve isovaleraldehyde and valeraldehyde completely within a reasonable amount of time. Although open tubular columns containing moderately polar stationary phases are available, columns coated with strongly polar stationary phases such as OV-275® are not commercially available due to problems associated with their immobilization. One of the highest polarity general purpose stationary phases available for GC is poly(biscyanopropylsiloxane). Although not currently available as a bonded phase, it has been used for an extended period for the analysis of fatty acid methyl esters, dioxins and aromatic compounds.[87] Other polar stationary phases which are not common in GC may find applicability in packed column SGC due to the added wettability of the porous particles.

Figure 2.5.3 shows chromatograms of standard aldehydes under conditions giving similar retention times. Temperature programming was used with the Supelco SP-2340 poly(biscyanopropylsiloxane) open tubular column, and isothermal SGC was used with the OV-275®-encapsulated particles. SGC was able to perform better separations under isothermal conditions than was GC using temperature programming. Since there is no cool-down step required for subsequent runs, SGC can provide a substantial time savings with equal or better performance than conventional GC for the separation of aldehydes. GC was not able to resolve isobutyraldehyde and butyraldehyde, while SGC was able to produce near-baseline separation under constant

temperature and pressure conditions. Using OV-275®-encapsulated particles, all of the aldehydes were resolved with relatively good peak shapes. Some tailing was observed for fast eluting peaks and is likely due to some dead volume in the SGC injector. For faster separations, the column was shortened by 20 cm, and the resulting chromatogram in Figure 2.5.4 shows a similar separation in approximately 60 s.

A number of advantages arise from using CO_2 as the mobile phase compared with lighter gases in GC. The solvating power of CO_2 allows for a significant increase in column efficiency per unit time and better retention using similar polar stationary phases. In gas chromatography, mobile phases such as helium and hydrogen offer little, if any, solvating power. Although the use of packed columns often results in large solute retention, the solvating power of CO_2 can offset this effect and produce high efficiency and fast separations. By using packed capillary columns with carefully chosen bonded stationary phases, selective separation of underivatized aldehydes can be achieved under SGC conditions. While bonded and crosslinked stationary phases are clearly most desirable, the experiments reported here illustrate the advantages of using a high cyanopropyl content phases for the separation of aldehydes in SGC. Since SGC can provide equal or better separation under isothermal conditions, significant time savings may be realized due to the elimination of a "cool-down" step normally required for temperature-programmed GC analyses.

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Table 2.1.1 Mass spectrometry data for resin acid methyl esters.[1]

I. Pimaric acid Me-ester, 316 $[M]^+$ (31), 301 (29), 257 (47), 241 (33), 180 (42), 121 (100).

II. Sandaracopimaric acid Me-ester, 316 $[M]^+$ (44), 301 (52), 257 (36), 241 (53), 180 (22), 121 (100).

III. Isopimaric acid Me-ester, 316 $[M]^+$ (28), 301 (35), 256 (54), 241 (100), 187 (18).

IV. Dehydroabietic acid Me-ester, 314 $[M]^+$ (12), 299 (21), 239 (100).

V. 7-hydroxydehydroabietic acid Me-ester (unknown diastereomer), 330 $[M]^+$ (5), 312 (32), 253 (10), 237 (100), 195 (43), 162 (19).

VI. 15-hydroxydehydroabietic acid Me-ester, 330 $[M]^+$ (14), 315 (73), 312 (10), 255 (100), 237 (30), 197 (23).

VII. 7-oxodehydroabietic acid Me-ester, 328 $[M]^+$ (25), 296 (20), 268 (20), 253 (100), 213 (21), 211 (23), 187 (19).

[1] Data obtained from references [7,15-19].

Table 2.1.2. Aerosol Components, Relative Percents ± Standard Deviation, by Integration of Total Ion Chromatograms.

Resin Acid (Methyl Ester)	[1]Unheated Flux	Heated Flux Extract	Heated Flux With Pb/Sn Solder	Heated Flux With Pb/Sn Solder, Prolonged Sampling	[4]Personal Sample
Pimaric Acid	8.1 ± 0.47	9.3 ± 0.53	8.6 ± 0.25	8.9 ± 0.70	18.7
Sandaraco-pimaric Acid	ND	1.0 ± 0.15	1.1 ± 0.19	1.0 ± 0.081	2.3
Isopimaric Acid	11.0 ± 0.93	10.1 ± 0.16	14.2 ± 1.21	11.4 ± 0.35	6.6
Dehydroabietic Acid	10.9 ± 0.91	12.8 ± 2.22	19.0 ± 1.47	17.4 ± 2.52	67.6
Abietic Acid	65.6 ± 1.86	64.3 ± 1.73	54.5 ± 0.10	58.7 ± 2.26	ND
[2]*Unknown Resin Acid*	3.4 ± 0.54	2.4 ± 0.53	2.5 ± 0.46	2.6 ± 0.51	ND
[3]*Oxidized Resin Acids*	ND	ND	ND	ND	4.8

[1]Unheated flux is from non-aerosolized parent material
[2]Unknown resin acid = likely $[m]^+$ of 316 *m/z*
[3]Oxidized resin acids = combined 7-hydroxy and 7-oxodehydroabietic acid methyl esters. 15-Hydroxydehydroabietic acid peak excluded as phthalate contaminant peak co-eluted with this compound.
[4]Personal sample is representative of several collected as described in *Materials and Methods.*

ND = peak height less than 1% of maximum peak

Table 2.3.1. Quantitative blank analysis for aldehyde oxazoladines. Mean integrated peak values for blanks divided by internal standard values± standard deviations by type of extraction, and comparison with rosin core solder sample prepared at 350 °C with internal standard at same concentration.

Aldehyde Derivative	**Toluene Extraction and Sonication**	**Methylene Chloride Extraction no Sonication**	**Rosin Core Solder Sample Methylene Chloride Extraction no Sonication**
Formaldehyde	0.169 ± 0.0075	0.385 ± 0.0565	1.55
Acetaldehyde	0.0519 ± 0.00258	0.0502 ± 0.00941	24.7
Benzaldehyde	0.775 ± 0.0933	0.540 ± 0.638	0.503
Unknown likely 7-carbon aldehyde oxazoladine	2.95 ± 0.542	1.590 ± 0.244	1.936

Table 2.5.1a. SGC Column Efficiencies at Constant Temperature or Constant Pressure.[a]

P_i (atm)[b]	T(°C)	u_{ave}[c] (cm s^{-1})	N	N_t (plates s^{-1})	k
150	150	1.79	60,000	181	2.7
190	150	2.13	52,000	232	2.1
250	150	2.59	43,000	290	1.5
170	90	2.01	29,000	86	3.3
170	130	2.05	46,000	169	2.5
170	170	2.00	55,000	241	1.9

Table 2.5.1b. SGC Selectivities at Constant Temperature or Constant Pressure.[a]

P_i (atm)	T (°C)	$\alpha_{1/2}$	$\alpha_{5/6}$	$\alpha_{9/10}$
150	150	1.1	1.2	1.2
210	150	1.1	1.1	1.1
250	150	1.2	1.1	1.0
		$\alpha_{2/3}$	$\alpha_{8/9}$	$\alpha_{10/11}$
170	70	1.4	1.3	1.5
170	110	1.4	1.3	1.4
170	170	1.4	1.2	1.3

[a] Conditions: 0.70 m x 250 μm i.d. fused silica capillary packed with 10 μm porous (80 Å) OV-275®-encapsulated silica, CO_2 mobile phase, FID.

[b] P_i = Pressure at column inlet

[c] u_{ave} = average mobile phase linear velocity measured using methane as an unretained marker.

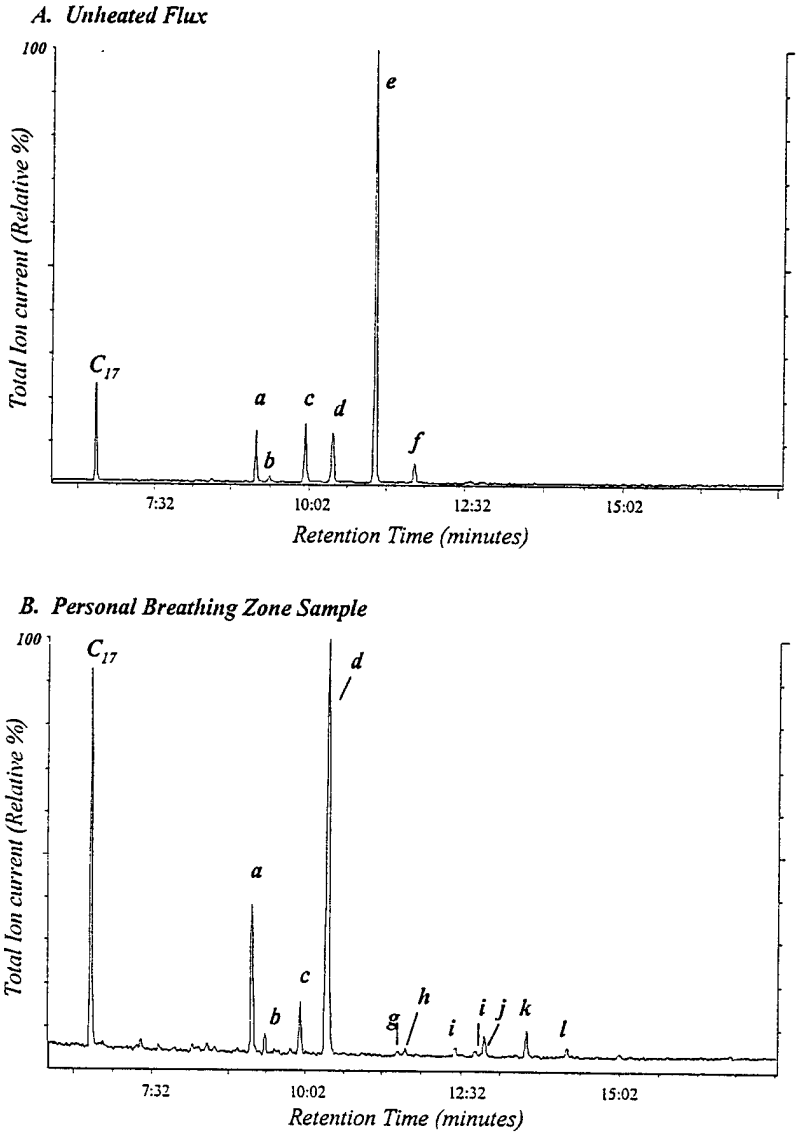
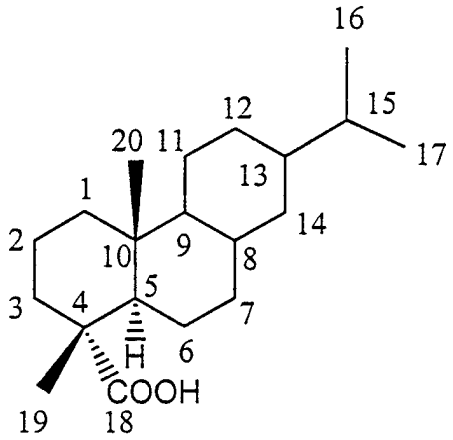
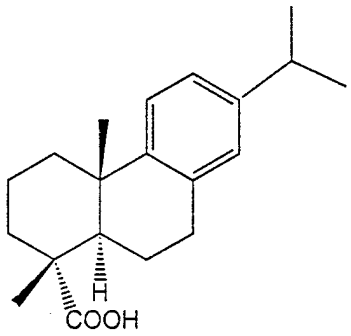
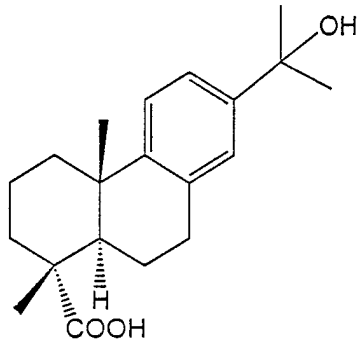


Figure 2.1.1. GC/MS chromatogram of unheated rosin flux extracted from (A) rosin core solder, and (B) personal breathing zone sample. Peaks identifications: C_{17}, heptadecanoic acid methyl ester (internal standard); *a*, pimaric acid methyl ester; *b*, sandaracopimaric acid methyl ester; *c*, isopimaric acid methyl ester; *d*, dehydroabietic acid methyl ester; *e*, abietic acid methyl ester; *f*, unknown resin acid methyl ester, probable 316 $[M]^+$; *g*, unknown resin acid methyl ester, probable 310 $[M]^+$; *h*, unknown resin acid methyl ester, probable 312 $[M]^+$; *i*, 7-hydroxydehydroabietic acid methyl ester; *j*, 15-hydroxydehydroabietic acid methyl ester; *k*, 7-oxodehydroabietic acid methyl ester; *l*, unknown resin acid methyl ester, probable 330 $[M]^+$.

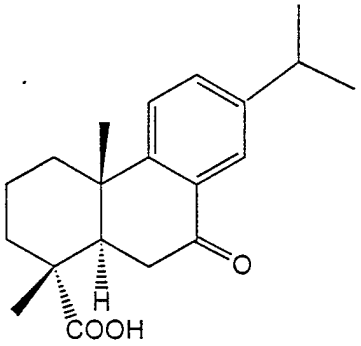




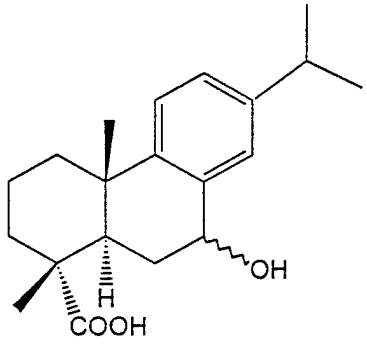
Dehydroabietic Acid



15-Hydroxydehydroabietic Acid



7-Oxodehydroabietic Acid



7-Hydroxydehydroabietic Acid

Figure 2.1.2. Abietane resin acid ring numbering system, dehydroabietic acid, and several oxidized dehydroabietic acid compounds of interest.

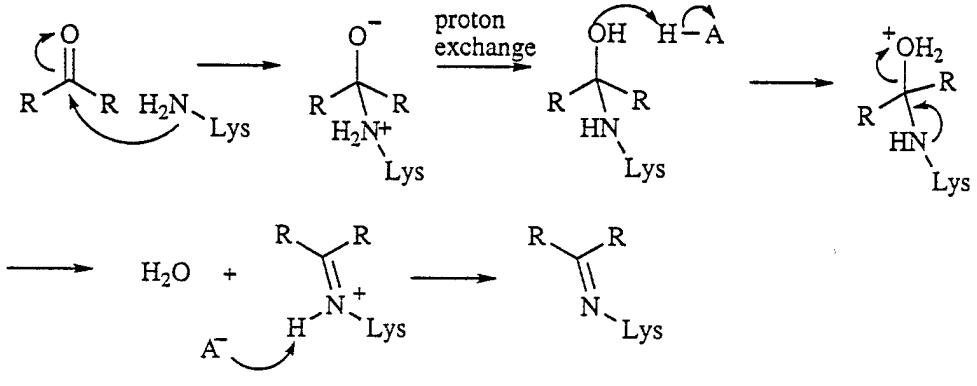


Figure 2.2.1. Proposed imine formation reaction scheme between 7-oxodehydroabietic acid and L-lysine.

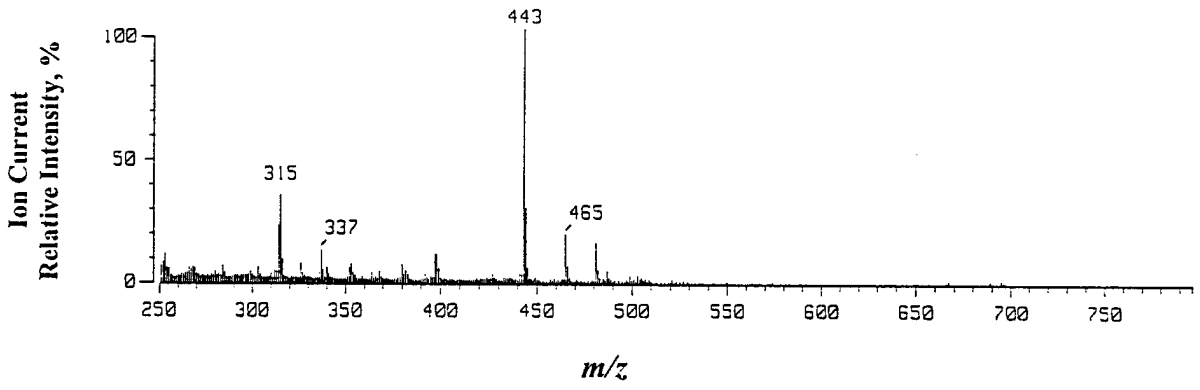


Figure 2.2.2. Mass spectrum of conjugate in benzene with base catalysis using fast atom bombardment mass spectrometry. Protonated 7-oxodehydroabietic acid $(M+H)^+$ peak is 315 *m/z*, diprotic resin acid/amino acid conjugate $(M+H)^+$ is 443 *m/z*, peaks at 337 and 465 *m/z* represent (acid + $Na)^+$ and (conjugate + $Na)^+$ respectively.

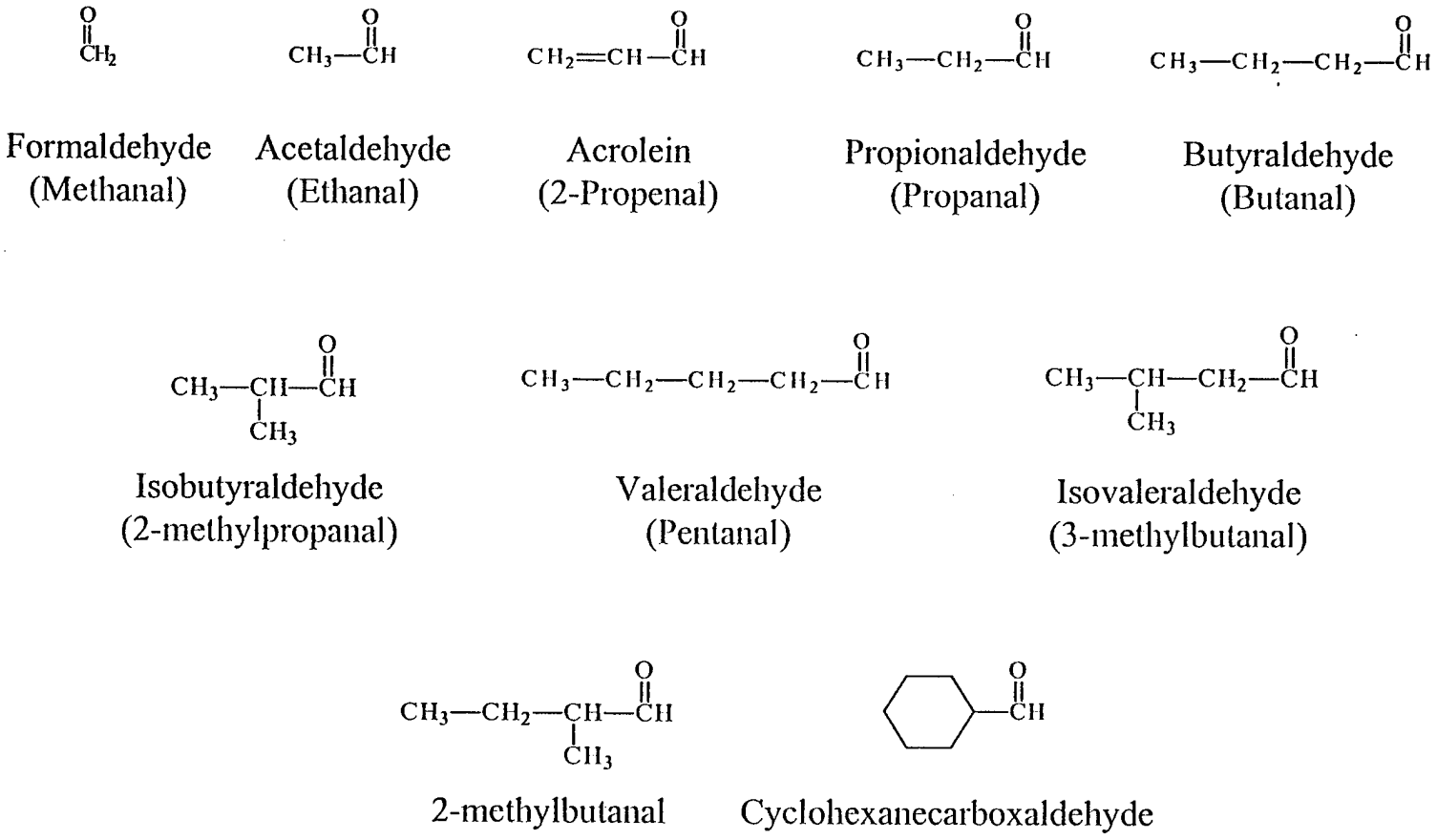
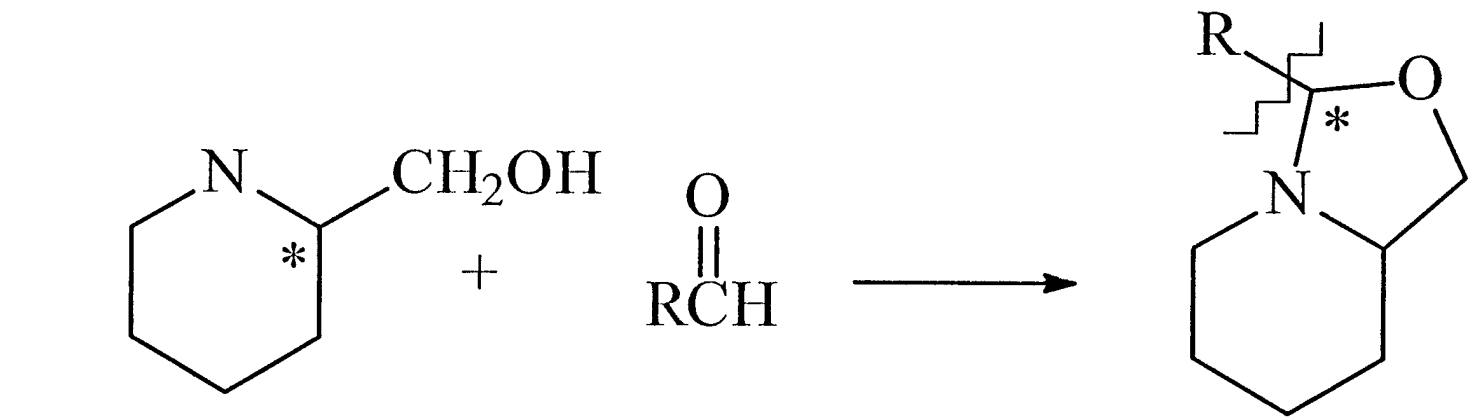


Figure 2.3.1. Chemical structures of selected aldehydes observed in samples created by heating rosin flux and rosin core solder.



2-hydroxymethylpiperidine

Oxazolidine Derivative

Figure 2.3.2. 2-Hydroxymethylpiperidine aldehyde derivatization scheme. Asterisk denotes stereocenter.

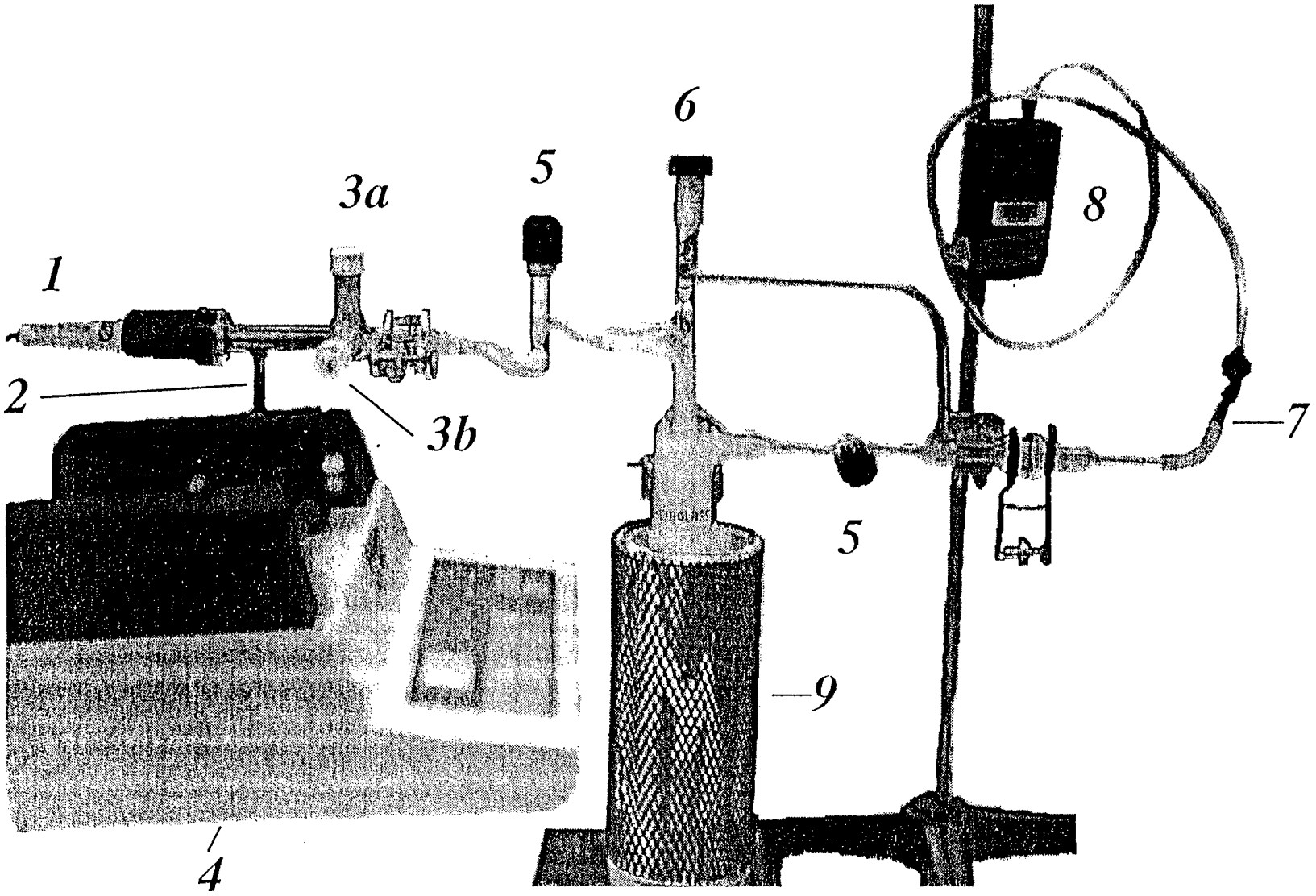


Figure 2.3.3. Sample collection apparatus. Element identifications: (1) Temperature-controlled soldering iron, (2) Inlet for dried air, (3a) Septum covered port for introduction of rosin core solder, (3b) Septum covered inlet for introduction of liquid rosin flux using controlled syringe pump, (4) Variable speed syringe pump, (5) Valves for sealing cold trap section following sample collection, (6) Pressure release safety valve to bypass sealing valve, (7) Sample collection tube, (8) Sampling pump with attached sampling train, (9) Vacuum-insulated reservoir for cryogenic material to cool the cold trap.

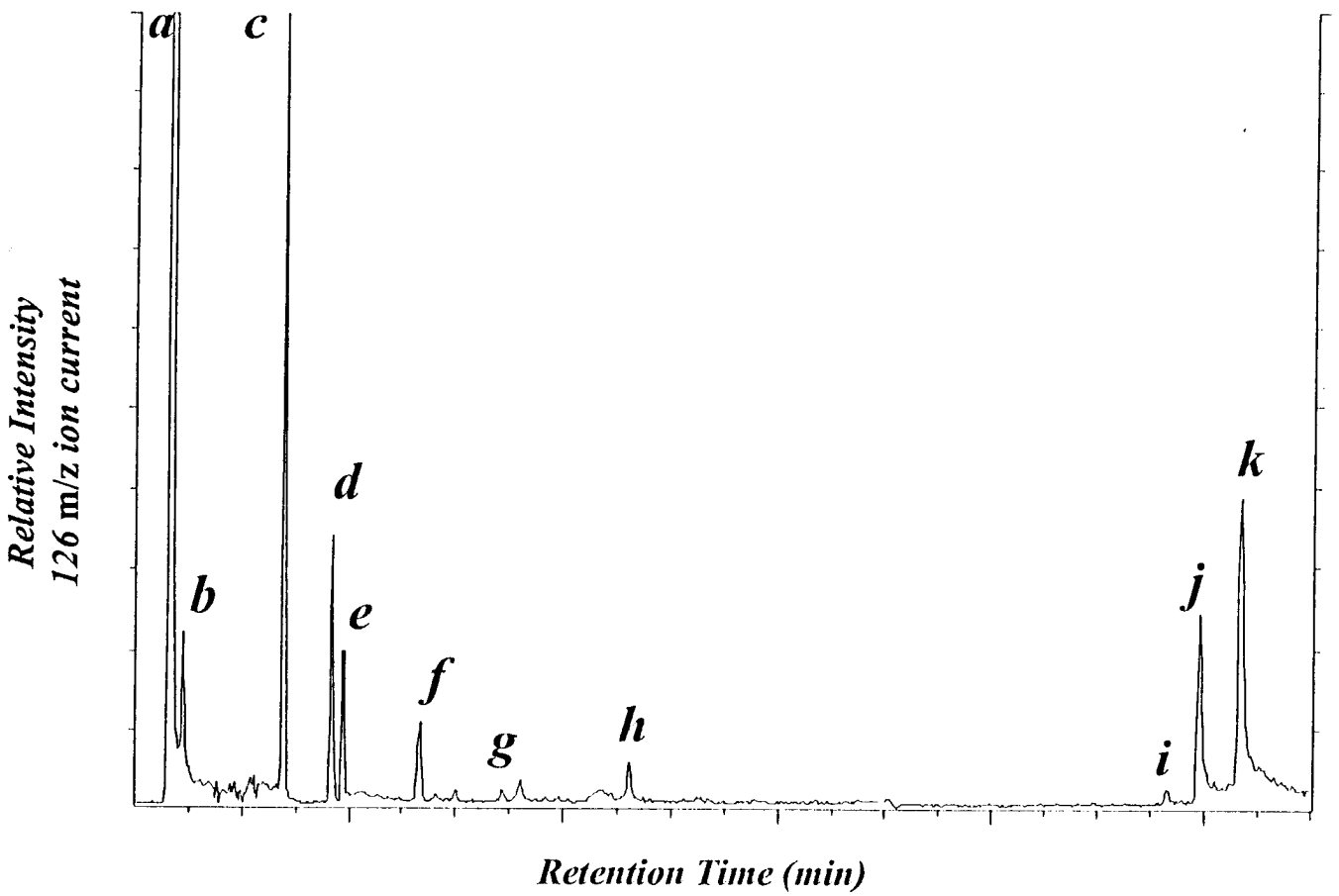


Figure 2.3.4. 126 *m/z* ion current chromatogram produced by sampling air after heating Kester rosin core solder (1m length) at 400 °C. Cryogenic trapping not used. Peak identifications (as oxazoladine derivatives): *a*) acetaldehyde, *b*) formaldehyde, *c*) acetaldehyde (2nd diastereomer of the derivative), *d*) propionaldehyde, *e*) acrolein, *f*) isobutyraldehyde, g) butyraldehyde, *h*) isovaleraldehyde, *i*) hexaldehyde, *j*) benzaldehyde, *k*) unknown probable 7-carbon aldehyde.

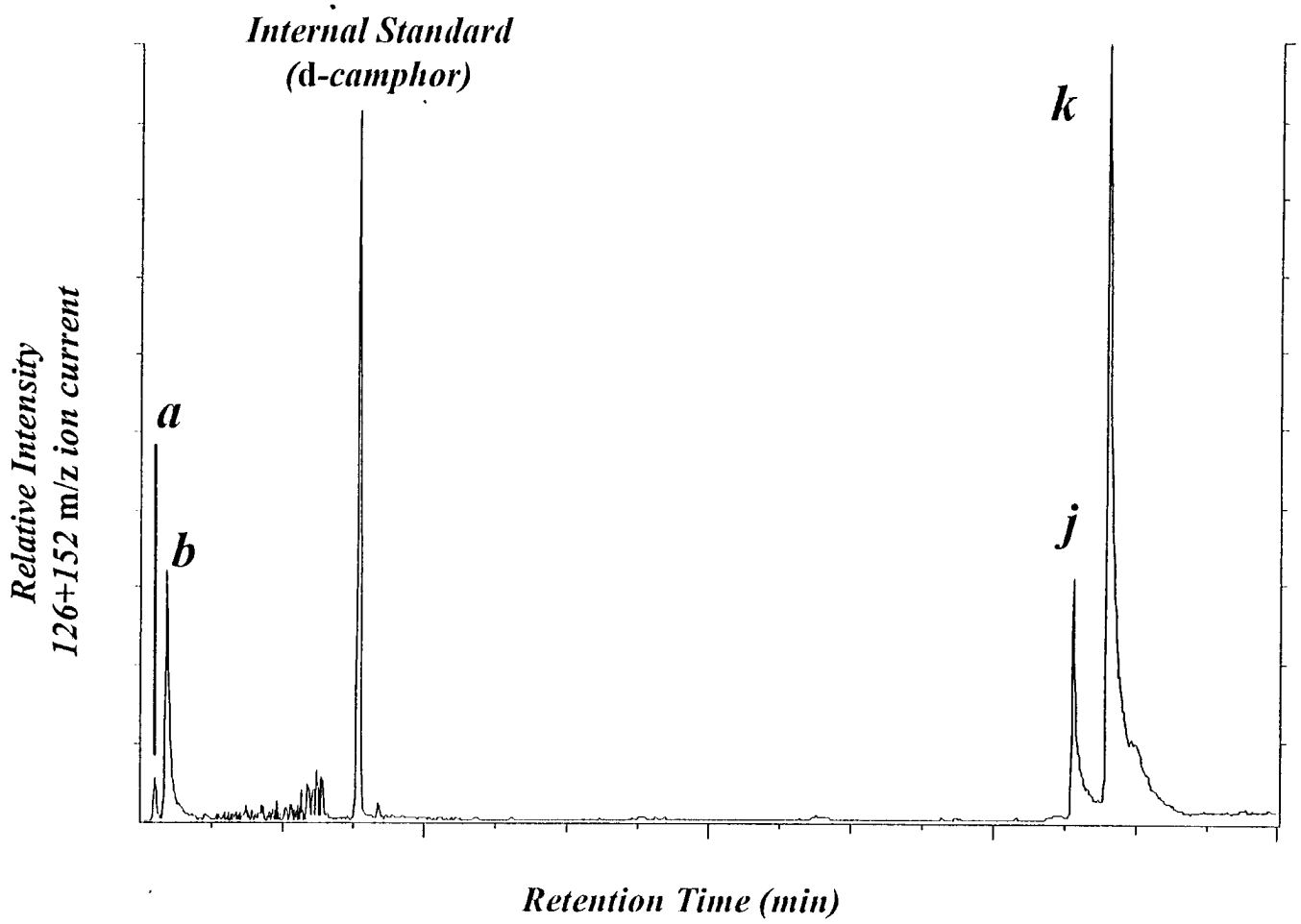


Figure 2.3.5. 126 + 152 *m/z* ion current chromatogram produced by extracting blank sampling tube with methylene chloride without sonication. Internal standard added. Peak identifications (as oxazoladine derivatives): *a*) acetaldehyde, *b*) formaldehyde, *j*) benzaldehyde, *k*) unknown probable 7-carbon aldehyde, 1 unit of unsaturation.

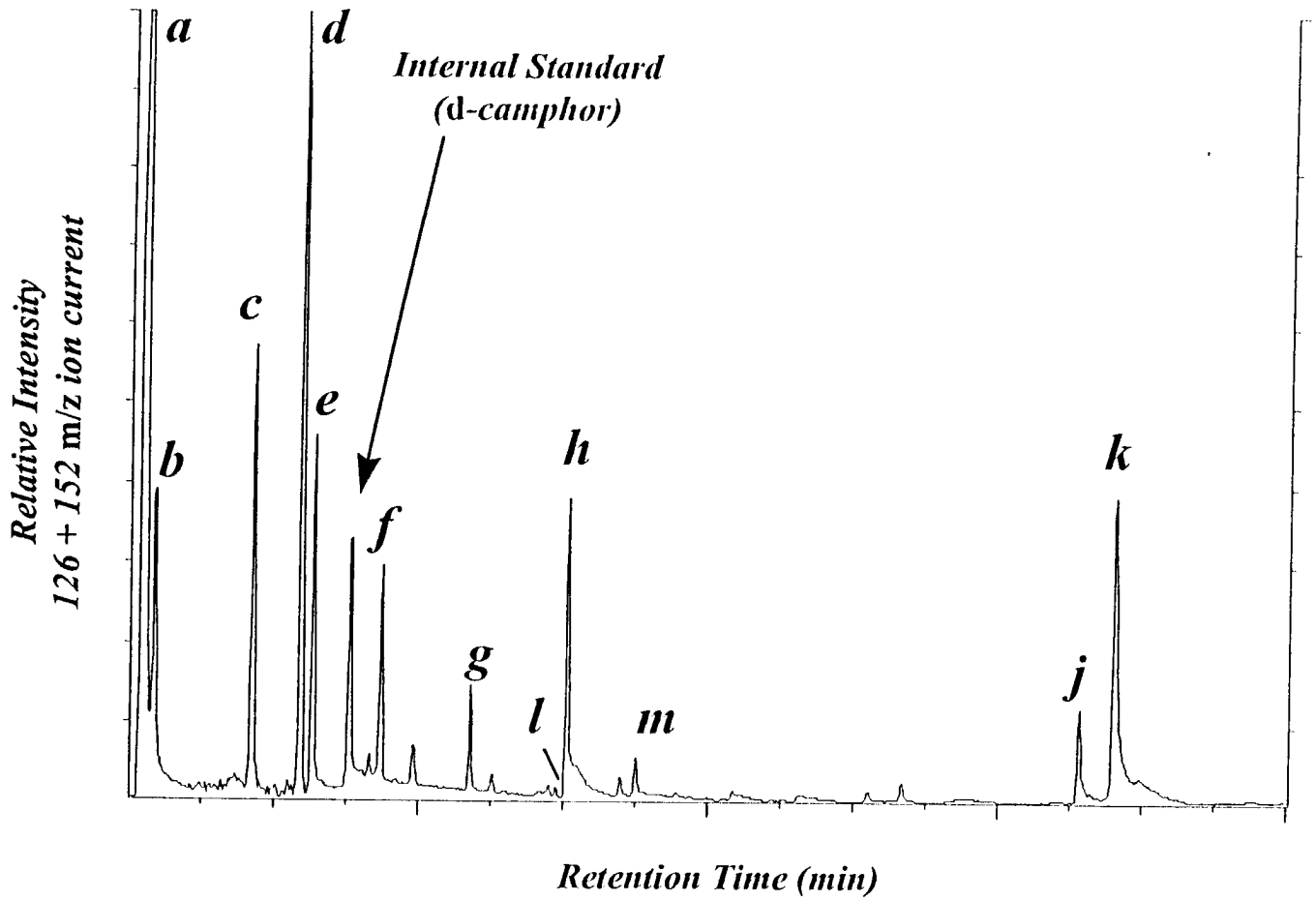
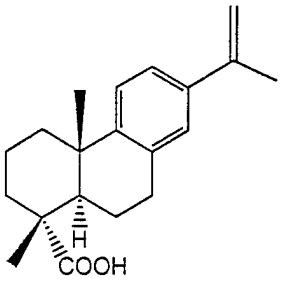
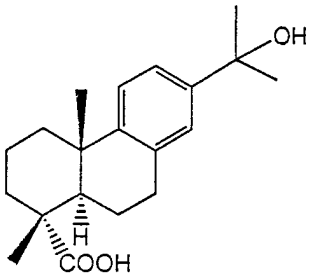


Figure 2.3.6. 126 + *152 m/z* ion current chromatogram produced by sampling air after heating Kester rosin core solder (1m length) at 350 °C. Cryogenic trapping not used. Internal standard added. Peak identifications (as oxazoladine derivatives): *a*) acetaldehyde, *b*) formaldehyde, *c*) acetaldehyde (2nd diastereomer of the derivative), *d*) propionaldehyde, *e*) acrolein, *f*) isobutyraldehyde, *g*) butyraldehyde, *h*) isovaleraldehyde, *i*) hexaldehyde, *j*) benzaldehyde, *k*) unknown probable 7-carbon aldehyde, 1 unit of unsaturation, *l*) 2-methylbutanal, *m*) valeraldehyde.



Abieta-8,11,13,15-tetraen-18-oic acid



15-Hydroxydehydroabietic acid

Figure 2.4.1. Resin acid metabolites observed from extracts from the *in vitro* metabolism system.

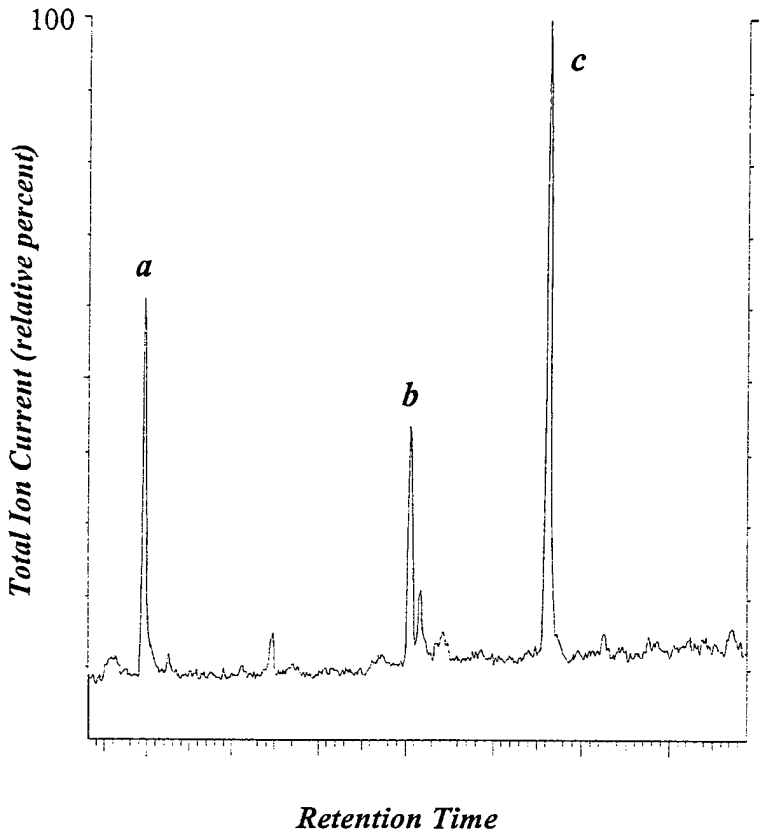


Figure 2.4.2. GC/MS chromatogram of *in vitro* metabolism system extract. Peak identification: ***a***, dehydroabietic acid methyl ester; ***b***, abieta-8,11,13,15-tetraen-18-oic acid methyl ester; ***c***, 15-hydroxydehydroabietic acid methyl ester.

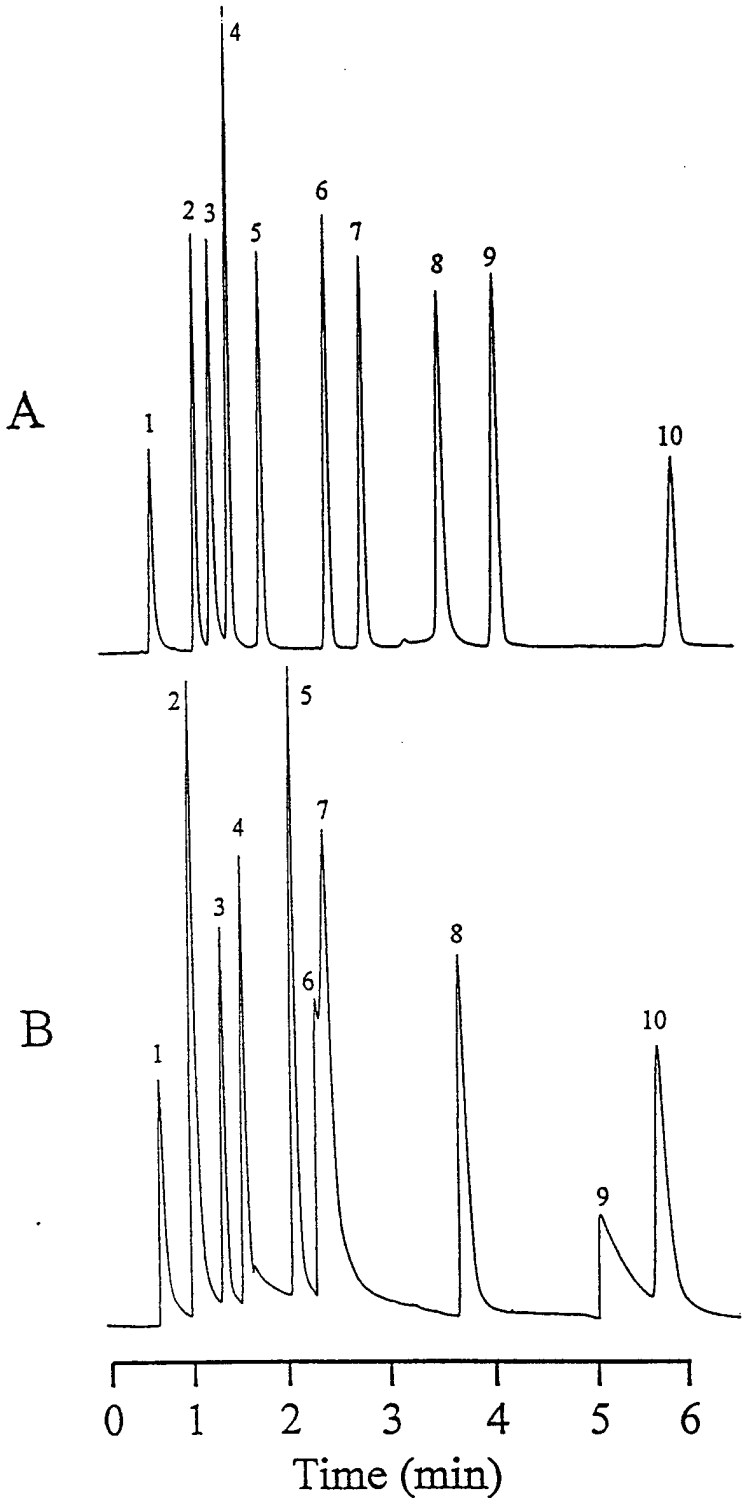


Figure 2.5.1. Separation of underivatized aldehydes using packed capillaries with (A) OV-275®-encapsulated silica and (B) ODS-bonded silica. Conditions: (A) 1.57 m x 250 μm i.d. capillary column packed with OV-275®-encapsulated particles (10 μm, 80 Å), CO_2, 190 atm, 150 °C, FID. (B) 30 m x 250 μm i.d. capillary column coated with SE-54 (0.25 d_f), He, 40 °C, FID. Peak identifications: (1) acetaldehyde, (2) propionaldehyde, (3) acrolein, (4) isobutyraldehyde, (5) butyraldehyde, (6) isovaleraldehyde, (7) valeraldehyde, (8) crotonaldehyde, (9) caproicaldehyde, (10) 2-furaldehyde.

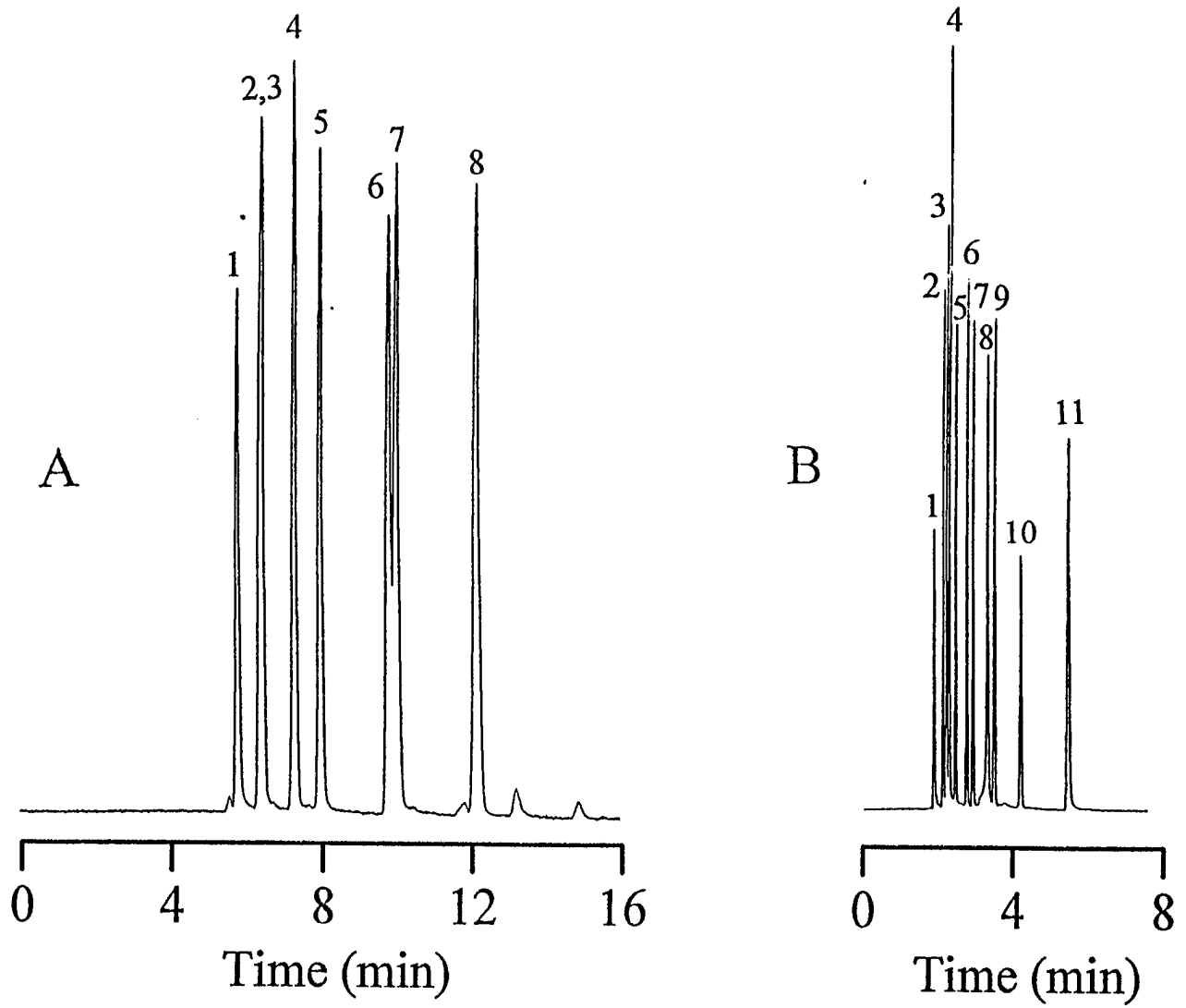


Figure 2.5.2. Separation of underivatized aldehydes using (A) SE-54 coated open tubular capillary column and (B) OV-275®-encapsulated silica packed capillary column. Conditions: (A) 1.57 m x 250 μm i.d. capillary column packed with OV-275®-encapsulated particles (10 μm, 80 Å), CO_2, 190 atm, 150 °C, FID. (B) 30 m x 250 μm i.d. capillary column coated with SE-54 (0.25 d_f), He, 40 °C, FID. Peak identifications: (1) acetaldehyde, (2) propionaldehyde, (3) acrolein, (4) isobutyraldehyde, (5) butyraldehyde, (6) isovaleraldehyde, (7) valeraldehyde, (8) crotonaldehyde, (9) caproicaldehyde.

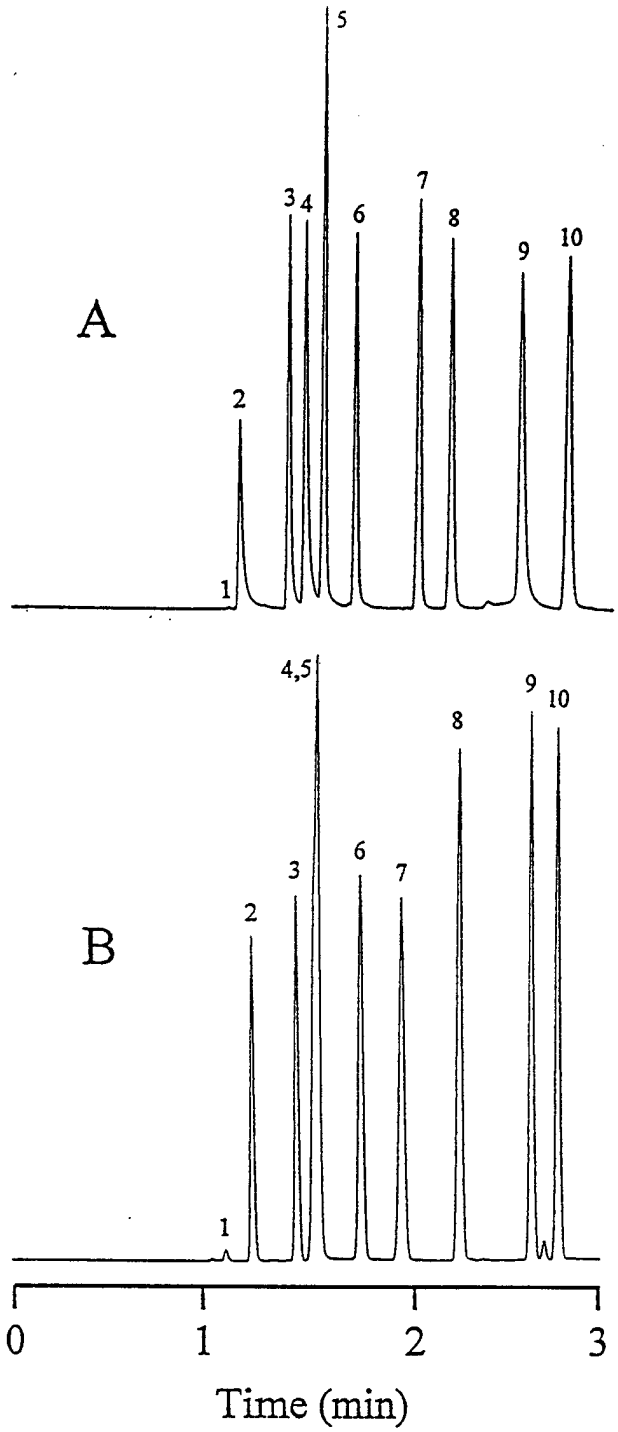


Figure 2.5.3. Separation of underivatized aldehydes using (A) OV-275®-encapsulated silica packed capillary column and (B) SP-2340 open tubular capillary column. Conditions: (A) 0.70 m x 250 μm i.d. capillary column packed with OV-275®-encapsulated particles (10 μm, 80 Å), CO_2, 170 atm, 150 °C, FID. (B) 15 m x 250 μm i.d. capillary column coated with poly(biscyanopropylsilxoane), 0.25 d_f, He, 40 °C for 1.5 min, 40-225 °C at 30 °C min^{-1}, FID. Peak identifications: (1) formaldehyde, (2) acetaldehyde, (3) propionaldehyde, (4) acrolein, (5) isobutyraldehyde, (6) butyraldehyde, (7) isovaleraldehyde, (8) valeraldehyde, (9) crotonaldehyde, (10) caproicaldehyde.

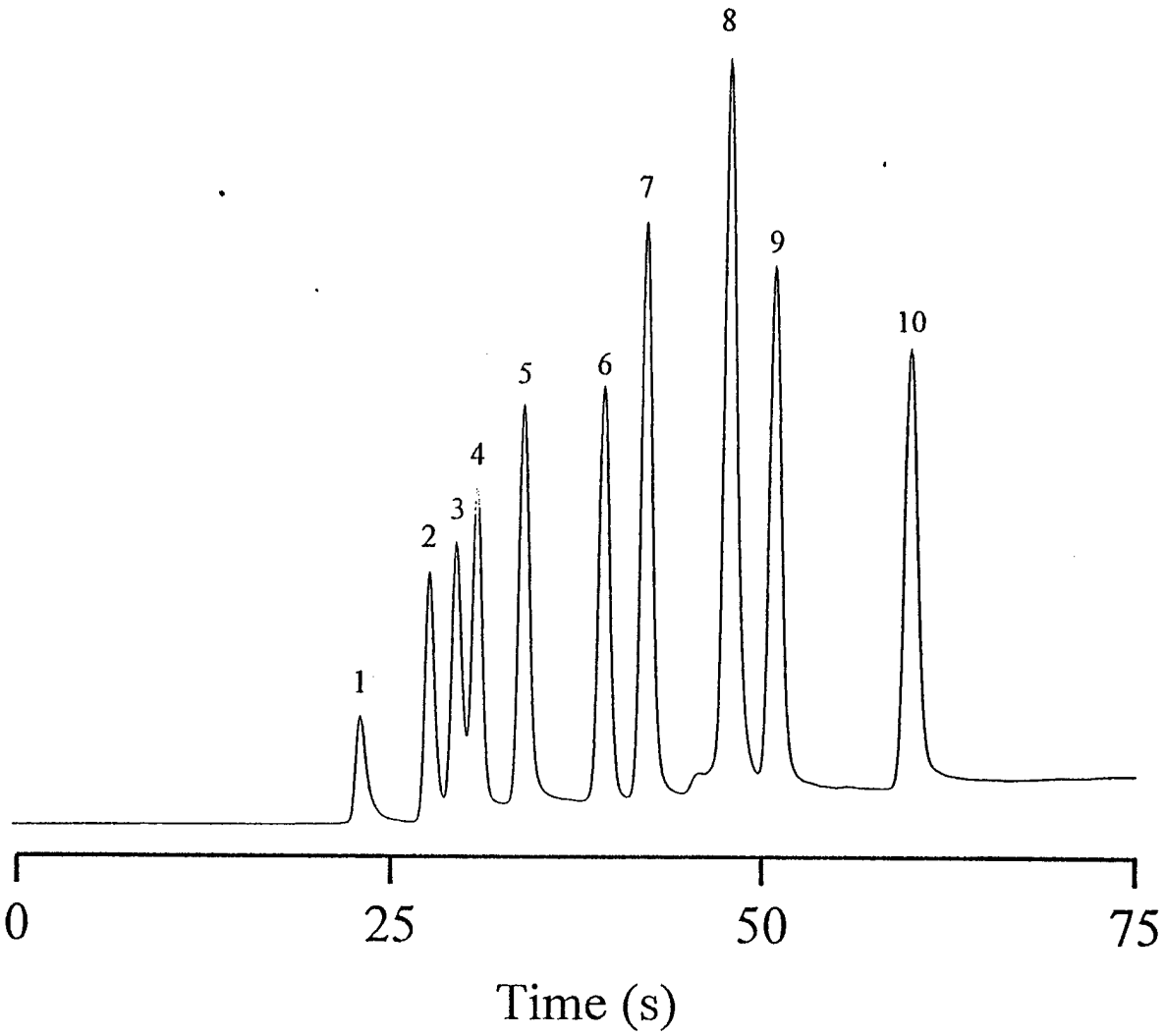


Figure 2.5.4. Fast separation of underivatized aldehydes using SGC. Conditions: 0.50 m x 250 μm i.d. capillary column packed with OV-275®-encapsulated particles (10 μm, 80 Å), CO_2, temperature program, 150 °C for 45 s, 150-180 °C at 40°C min^{-1}, pressure program, 100 atm for 30 s, 100-180 atm at 40 atm min^{-1}, FID. Peak identifications: (1) acetaldehyde, (2) propionaldehyde, (3) acrolein, (4) isobutyraldehyde, (5) butyraldehyde, (6) isovaleraldehyde, (7) valeraldehyde, (8) crotonaldehyde, (9) caproicaldehyde, (10) heptaldehyde.

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13. ABSTRACT ***(Maximum 200 words)***

Exposure potential for rosin-derived compounds, including volatile and semivolatile organic compounds produced during electronics soldering operations using rosin-based fluxes and rosin core solders, was investigated. A reaction system was constructed for trapping emission products. Particulate matter was collected on filters, and volatile organic compounds were collected on sorbent tube media for analysis by gas chromatography/mass spectrometry (GC/MS). Comparisons were made between reaction system samples and actual breathing zone samples collected during soldering operations.

Other work included: (1) A protein conjugation/haptenation mechanism for 7-oxodehydroabietic acid, an oxidized resin acid compound present in aerosol produced by heating rosin flux, (2) Identification of low molecular weight aldehydes in soldering emissions using gas chromatography/mass spectrometery (GC/MS), (3) *In vitro* studies of resin acid metabolism to identify mammalian metabolites produced from dehydroabietic acid, and (4) A rapid method for analyzing headspace sampling of aldehydes using solvating gas chromatography (SGC).

This project provided information regarding the specific compounds generated during rosin heating in electronic soldering applications. A possible biological mechanism was shown for sensitization to a resin acid present in aerosol produced during heating rosin flux. The work described may prove useful in producing an exposure standard for contaminant sampling during electronics soldering operations.

14. SUBJECT TERMS
SOLDERING, ROSIN, FLUX, MICROMINIATURE REPAIR, ALDEHYDE, RESIN ACID, SOLVATING GAS CHROMATOGRAPHY, PACKED CAPILLARY COLUMN

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